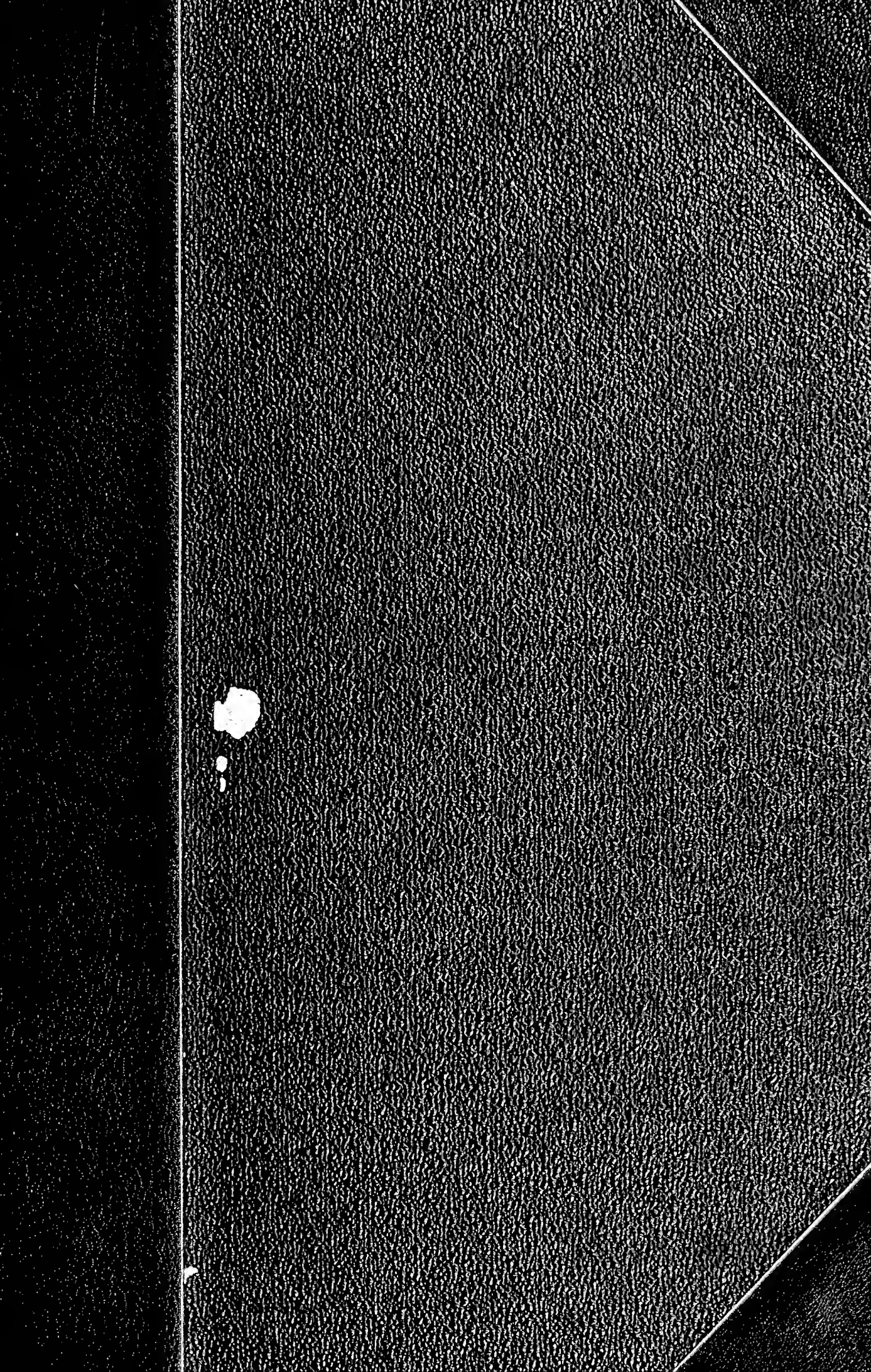

PARASITOLOGY

CAMBRIDGE UNIVERSITY PRESS
London: FETTER LANE, E.C.
C. F. CLAY, Manager

Edinburgh: 100, PRINCES STREET
London: H. K. LEWIS, 136, GOWER STREET, W.C.
WILLIAM WESLEY AND SON, 28, ESSEX STREET, STRAND
Berlin: A. ASHER AND CO.
Leipzig: F. A. BROCKHAUS
Chicago: THE UNIVERSITY OF CHICAGO PRESS
Bombay and Calcutta: MACMILLAN AND CO., Ltd.

PARASITOLOGY

A SUPPLEMENT TO THE

JOURNAL OF HYGIENE

EDITED BY

GEORGE H. F. NUTTALL, F.R.S.
Quick Professor of Biology in the University of Cambridge

AND

A. E. SHIPLEY, F.R.S.
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ASSISTED BY

EDWARD HINDLE, PH.D.

Volume V 1912–13

Cambridge
at the University Press
1913

Cambridge:
PRINTED BY JOHN CLAY, M.A.
AT THE UNIVERSITY PRESS

Vol. 5, No. 3 September, 1912

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AND WILLIAM WESLEY AND SON, 28, ESSEX STREET, STRAND
EDINBURGH: 100, PRINCES STREET
BERLIN: A. ASHER & CO.
LEIPSIC: BROCKHAUS
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CONTENTS

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VOLUME V SEPTEMBER, 1912 No. 3

FANNIA[1] (*HOMALOMYIA*) *CANICULARIS* LINN. AND *F. SCALARIS* FAB.

AN ACCOUNT OF THE BIONOMICS AND THE LARVAE OF THE FLIES AND THEIR RELATION TO MYIASIS OF THE INTESTINAL AND URINARY TRACTS.

BY C. GORDON HEWITT, D.Sc.,
Dominion Entomologist, Ottawa, Canada.

(With Plate VII, and 7 Text-figures.)

THE two flies *Fannia canicularis* Linn. and *F. scalaris* Fab. are, on account of their habits, of considerable economic importance in their relation to man. It is therefore desirable that those engaged in public health and medical work and others should have a knowledge of the breeding and other habits of these flies, which they are certain to meet in their work under circumstances of varying importance. The inquiry of which this account is the result was undertaken several years ago at the request of Dr Monckton Copeman in connection with the Local Government Board's inquiry on the carriage of infection by flies in the reports of which a portion of this paper has been included. Owing to my removal from England to Canada in 1909 and subsequent pressure of work, its completion was delayed.

These two species of flies belong to the dipterous family Anthomyidae, many of which resemble the house-fly (*Musca domestica*) in appearance. They are characterised chiefly by the close approximation of the eyes of the male, the comparatively large squamae, or lobes, on the posterior sides of the bases of the wings, and the open first posterior, or apical, cell (5R) of the wing. Most of the larvae, or maggots, feed upon decaying vegetable or animal substances.

[1] By the rules of priority the generic name *Fannia* of Robineau Desvoidy 1830, which he gave in his *Essai sur les Myodaires*, will have to replace Bouche's genus *Homalomyia* to which genus these species are usually referred but which was not created until 1834.

Without close examination the two species under examination are liable to be mistaken for the same species, but such an examination will serve to separate them. The abdomens of both species are conical, but the basal segments of the abdomen of *F. canicularis* are partially translucent and the abdomen of *F. scalaris* is black overspread with bluish grey; the mid-tibiae of the latter species bear a distinct tubercle which is not found in *F. canicularis* (Fig. 3).

Fannia canicularis L.

(*The Lesser House-fly.*)

This species (Plate VII) is the less common of the two species of flies found in houses. Its occurrence and frequency are, however, very variable and no valid explanation has been found so far in my investigations to account for this variability. *F. canicularis* is more abundant than *M. domestica* for a short time during the early part of the summer, usually in May and June. With the beginning of the hot weather the numbers of the latter increase enormously and replace the Lesser House-fly. In many cases which were observed the latter seemed to retreat in small numbers to the rooms of the house not devoted to cooking and they may be frequently found flying in a characteristic, jerky and hovering manner around chandeliers, etc. in the living and bed-rooms. In country houses, however, they frequently occur in numbers in the kitchens, as an examination of fly traps and papers in such places indicates.

The numerical abundance of *F. canicularis* in comparison with the abundance of *M. domestica* varies considerably. In a collection of nearly 4000 flies made in different situations, such as kitchens, restaurants, bed-rooms, etc. in 1907, this species formed 11·5 per cent. of the total number. In 1900 Howard found that in Washington, U.S.A. only about 1 per cent. of a collection of over 23,000 flies made in rooms where food was exposed were *F. canicularis* and over 98 per cent. were *M. domestica.* Hamer, in 1908, in collections made in kitchens and "living rooms" of houses near depots for horse manure in London found that the percentage of *F. canicularis* varied from 17 per cent. to 24 per cent. Niven gives the results of collections made at six different stations in Manchester. The total number of flies caught was 8553, of which 8196 were *M. domestica,* 293 *F. canicularis,* and 64 were other species. Thus, *F. canicularis* constituted 3·4 per cent. of the total fly population. Robertson gives the results of similar collections made in


NATURAL HISTORY

Fannia canicularis ♂. ×10.

Birmingham where, of 24,572 flies caught, 91 per cent. were *M. domestica* and 4·7 per cent. *F. canicularis*. From observations that I have made in many localities in different neighbourhoods, I do not think that this species would often form more than 25 per cent. of the total fly population. After *M. domestica*, however, it is the next fly of importance inhabiting houses and well deserves the title of Lesser House-fly. It is known in Germany as "die kleine Stubenfliege." The specific description of this species is as follows:

Male. Head iridescent black, silvery white especially around the eyes. The antennae are blackish grey with a non-setose arista shown in the figure (Plate VII). Palps black. The thorax is blackish grey with three indistinct black longitudinal stripes; the scutellum is grey and bears long setae; the sides of the thorax are lighter. The abdomen consists of five visible segments. In the male it is somewhat parallel-sided and possesses three and sometimes four pairs of golden translucent areas situated laterally in the proximal region. The legs are black and the middle femora bear comb-like setae below (Fig. 3). The somewhat large squamae at the bases of the wings are white and the halteres are yellow. Length 5·5 mm.

Female. The head of the female is grey with a wide frons, black frontal stripe and grey sides. The longitudinal stripes of the thorax are faint and the abdomen, which is more pyriform than that of the male, has a slightly golden attachment.

Proportion of sexes. Great disparity in the proportion of males to females is found in this species as it occurs in houses. Hamer showed in 1909 that the males constitute from 75 to 85 per cent. of the total flies of this species caught in balloons and on fly papers. This, however, does not indicate a disparity in the proportion of males to females in the species, as I have found that the females are more commonly found out-of-doors, especially in the neighbourhood of the breeding places.

Breeding habits. The breeding habits of this species are somewhat similar to those of the House-fly, *M. domestica*. The larvae breed in decaying and fermenting vegetable and animal matter and also in excrementous matter. In 1848 Heeger recorded it as living in the caterpillars of *Epischnia canella*; Roth found them in the nest of the humble bee, *Bombus terrestris*, and Schiner observed them in the bottom of a box in which a dormouse had been kept. Taschenberg also records the larvae as being found in snails, in old cheese and in pigeon nests; he reared

the flies from sugar beet and Brischke found the larvae in the stalks of rape. I have found them commonly in human excrement and in a variety of decaying vegetable substances, even in rotting grass. In England they may be found in the larval stages from May to October. Howard has reared them from human excrement during the same period in the United States. The eggs are white and cylindrically oval.

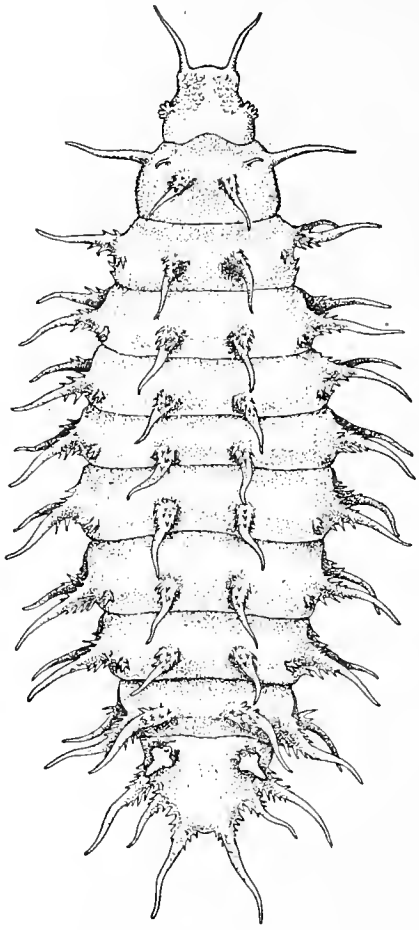

Fig. 1. *Fannia canicularis*. Larva. ×17.

Larva. The larva of *F. canicularis* (Fig. 1) is wholly different from that of *M. domestica*; its body being provided with a number of appendages or spiniferous processes. These are arranged in three pairs of longitudinal series and there are in addition two pairs of series of smaller processes.

The body is compressed dorso-ventrally and the surface is roughened in character and in places spiniferous. It consists of twelve segments,

of which the first, or pseudocephalic segment, is often withdrawn into the second or prothoracic segment, as shown in the figure. The posterior end of the body is very obliquely truncate. The full-grown larva measures 5 to 6 mm. in length. The three series of pairs of spiniferous flagelliform processes, or appendages, are arranged as follows: A dorsal

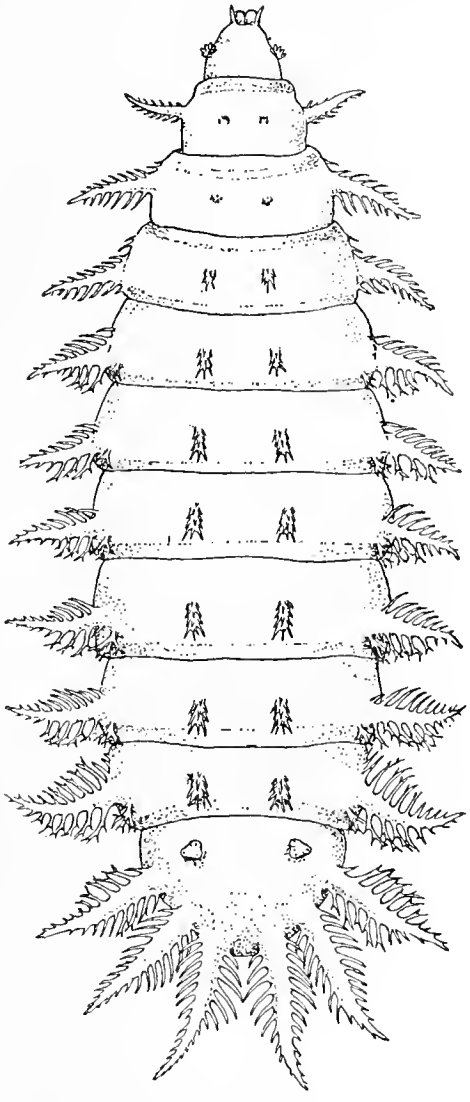

Fig. 2. *Fannia scalaris*. Larva. ×12.

series consisting of ten pairs of processes commencing with an antenna-like pair of processes at the anterior border of the prothoracic segment (segment II) and slightly increasing in size posteriorly. A latero-dorsal series of ten pairs of processes which commences on segment III and is continued to the posterior end of the body. A latero-ventral series

which commences on segment III and is continued posteriorly. These flagelliform processes are spiniferous, the spines being well developed at the basis of the processes and gradually decreasing in size distally. The twelfth or anal segment is provided with three pairs of these processes of unequal size; the most anterior pair is the longest on the body and the intermediate pair is shorter.

There is a series of pairs of small, almost sessile branched appendages (Fig. 5) situated near and slightly posterior to the bases of the latero-dorsal appendages. These were described by Kieffer. Each of these processes has three to four branches and they carry a small nucleiform organ which Chevril (1909) has also described. He believes that this organ is of the nature of an exuvial gland and correspondent to Verson's gland.

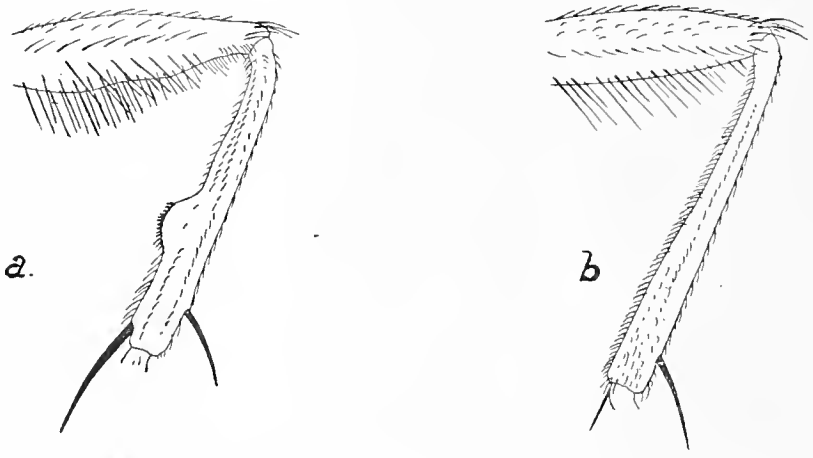

Fig. 3. Median joints of middle pair of legs (right), posterior aspect.
a. Fannia scalaris. *b. Fannia canicularis.*

On the ventral surface of the body and extending posteriorly from segment III there is to be found a series of pairs of small spiniferous papillae. Between these there is on each segment a transverse row of four groups of spines.

The anterior, or prothoracic spiracular processes (Fig. 4 *a. sp.*) have usually seven finger-like lobes, though the number may vary from five to eight, and between the second and third lobes there appears to be a small stigmatic organ. The posterior spiracular processes have a trilobed appearance, but a close examination reveals their four-lobed character shown in Fig. 6; a stigmatic orifice is situated at the extremity of each lobe.

The spiny character of the flagelliform appendages and body of the larva causes particles of dirt to adhere readily to the bodies and

appendages of the larvae. In consequence the larvae have a very dirty appearance and their external features are almost hidden by the accumulated particles of dirt and filth adhering to them.

The larval period may extend over a week or it may last for three or four weeks if the substances in which the larvae are feeding become rather dry. When fully grown it is covered fairly thickly with dirt, which is of great assistance in the formation of the pupal case, as this is formed of the larval skin.

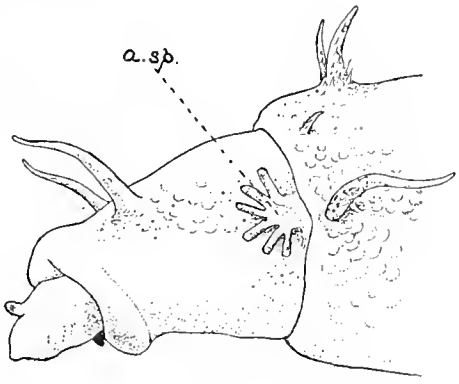

Fig. 4. *Fannia canicularis*. Larva. Lateral view of cephalic region; *a.sp.* anterior spiracular process.

Fig. 5.

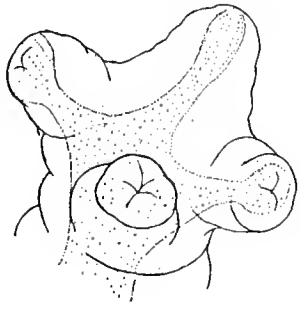

Fig. 6.

Figs. 5, 6. *Fannia canicularis*. Larva.

Fig. 5. Palmate and sessile dorsal appendage. Fig. 6. Posterior spiracular process.

In changing into the pupa, the cephalic region is retracted and the length of the larva is thereby decreased. The larval skin, with its covering of dirt particles, forms the co-arctate pupal case. Before pupating the larva leaves the very moist substance in which it may have been living and seeks a dryer situation. The pupal period extends over a period of seven to twenty-one days, or longer, and it

is not unlikely that larvae, which have developed very late in the season, pass the winter in the pupal state, as is the case in certain other species of Anthomyid flies. The adult fly emerges by pushing off the anterior segments of the pupal case.

F. scalaris Fab.

(The Latrine Fly.)

This species which, on account of its most common breeding habits, may be called the Latrine Fly, is very common both in European countries and in North America. Owing to its general similarity, it is often confused with the Lesser House-fly, *F. canicularis*, but the chief differences have already been indicated.

The description of the adult of *F. scalaris* is as follows:

Male. The frontal triangle on the head is black and is continued as a thin line to the vertex, being bordered on each side by a silvery white stripe. The antennae and palps are black. The thorax and scutellum are black and somewhat polished; the humeri are light-coloured. The abdomen is black, overspread with bluish grey and has a darker median stripe from which dark transverse bands arise forming by their junction with the median stripe black triangular markings. The legs are black and the middle femur is swollen ventrally, bearing on its broader side a group of brush-like bristles as will be seen from Fig. 3. The middle tibia is provided, as shown, with a distinct tubercle near the distal end.

Female. The colouring is more distinctly grey with a faint longitudinal striping on the thorax; the transverse markings on the abdomen are also indistinct. The head is grey with a wide frons.

F. scalaris is slightly larger than *F. canicularis*, measuring up to 6 mm. in length.

The habits of this species are somewhat similar to those of *F. canicularis* but it prefers excrementous matter as a nidus for the eggs and is very commonly found breeding in human excrement. It has been recorded breeding in human excrement by Schiner, Taschenberg, Howard and Newstead and I have also bred it from this material in England and Canada, both in privies where the excrement was found in a semi-liquid condition and on rubbish tips or dumps where it was mixed with ashes or clinker. Swammerdam figured what would appear to be the larva of this species as breeding in human excrement. Taschenberg

also refers to its breeding in mushrooms. In 1908 Dr David Sharp submitted the larva of this species to me for examination. He had found it in rotting fungus in the New Forest in September 1905 and noticed its similarity to Swammerdam's Latrine Larva.

The larvae emerge as early as eighteen hours after the deposition of the eggs and become full-grown in six to twelve days. The shortest time which I have recorded for the pupal stage was nine days, which was in the month of August, but I believe that under very favourable conditions the pupal stage would be passed in a shorter time.

Larva. The larva of this species (Fig. 2) has a general resemblance to that of *F. canicularis*, but a closer examination will reveal very marked differences and a number of distinguishing characters. In shape it is similar to the larva of *F. canicularis*, being compressed dorso-laterally. The appendages, or processes, however, are very different. The pair of antenna-like processes at the anterior and upper edge of

Fig. 7. *Fannia scalaris*. Larva. Ventral side of segment VII.

the prothoracic (second) segment are much shorter than those of *F. canicularis*, as will be seen from the figure, where they are shown dorsal to the oral lobes. On the dorsal side of the larva, from segment III to segment XI, is a series of nine pairs of short and somewhat thick processes of a very spiny character; the first two pairs being little more than spinous tubercles. As the processes of the third segment differ from the succeeding segment, they may be mentioned separately. There is a pair of latero-dorsal processes bearing spines. Ventral and slightly anterior to the base of each of these processes is a small spiniferous papilla. A short spinous latero-ventral appendage is situated slightly more posteriorly. Viewed from above the larva is seen to be surrounded by a fringe of feather-like processes. Segments IV to XI are each provided with a pair of pinnate latero-dorsal processes which gradually increase in size posteriorly. Three pairs of these pinnate processes surround the obliquely truncate dorsal surface of the twelfth segment. Situated laterally and ventral to the series of pinnate processes is a series of latero-ventral processes which are spinous

(as shown in Fig. 7) but much less pinnate and shorter than the latero-dorsal series. The latero-ventral processes of segment XII are situated more ventrally than those of the preceding segments and their usual place is taken by a small group of spines. Posterior to the base of each of the latero-dorsal processes of segments V to XI is a small branched process.

On the ventral side of the larva, extending from segments IV to XI, there is a series of pairs of small spiniferous papillae, as shown in Fig. 7, each of which is situated at the end of a transverse row of spines. Posterior to this transverse row of spines there is a shorter row of spines, divided into four groups. The anterior, or prothoracic, spiracular processes are six to eight-lobed; the usual number of the lobes being seven. The posterior spiracular processes are very similar to those of *F. canicularis*. Vogler (1900), who has given a good description of this larva, illustrates the anterior spiracular processes with eight lobes, and his figure of one of the posterior spiracular processes is not very clear.

The feathery character of the processes of *F. scalaris* is probably associated with the fact that the larvae usually live in substances of a semi-liquid character where such processes will be more advantageous than those of *F. canicularis* for life in such a medium. It may be of interest to note in this connection that the spiniferous and branched lateral appendages of the larvae of the genus *Fannia* were considered by Walsh (1870), and probably by other entomologists, to be 'branchiae' or gills. Walsh (*l. c.*) stated: "The larvae...wallow in moist decaying matter, whether animal or vegetable; and as in such situations they would be sometimes stifled for want of air, if they breathed through the spiracles or breathing holes with which all air-breathing insects are supplied, nature has replaced the spiracles by lateral 'branchiae,' or gills, by means of which they are able, after the manner of a fish, to extract the air from the fluids around them," and he compares them to the gills of the Ephemerid larvae.

Prior to pupation the larva leaves the moist situation for one of a drier character and the pupation is similar to that of *F. canicularis*.

F. scalaris is more commonly found than *F. canicularis* as the cause of intestinal myiasis and it also breeds more commonly in human excrement. These facts make its economic relation to man one of not a little importance.

The Relation of F. canicularis *and* F. scalaris *to Myiasis of the Intestinal and Urinary Tracts.*

For many years cases have been recorded of the presence of dipterous larvae or "maggots" in the human intestine, from which they have been expelled either by vomiting or through the anus. Their presence in the human body has frequently resulted in more or less serious intestinal or urinary troubles. The results of these cases are widely scattered through medical and other scientific journals and reference will be made to a number of the more important and typical cases.

Occurrence in the Alimentary Tract.

The presence of these larvae in the stomach is usually indicated by nausea, vertigo and violent pains; the larvae in many cases are expelled by vomiting. If they occur in the intestine, they are expelled with the faeces and their presence is signalized by diarrhoeal symptoms, abdominal pains, or haemorrhage caused by the traumatic lesions of the mucous membrane of the intestine which the larvae effect.

Rudolphi (1808) mentions the occurrence of insect larvae in the human body.

In 1839 Jenyns recorded the case of a clergyman of about 70 years of age, who complained of general feebleness, loss of appetite and a disagreeable epigastric feeling of a tremulous character. These symptoms began in the spring of 1836 and it was not until the autumn that the larvae were observed. They were expelled repeatedly in large numbers and their expulsion in this manner continued for several months. The larvae were about equal in size and extremely active on their appearance. The malady did not recur and the evacuation of the larvae ceased shortly; the patient's health gradually improved but not completely. The author calls attention to the fact that the symptoms made their appearance in the spring but the larvae were not expelled until the summer and autumn following. It would appear, therefore, that they entered the stomach in the egg state and after hatching passed into the intestine where they completed their growth.

The occurrence of dipterous larvae in the intestine is recorded by Hope (1840). Laboulbène (1856) records the rearing of the larvae belonging to this genus from the intestine of a woman who had suffered for some time from stomachic pains with loss of sleep and appetite. On October 12th she took castor oil, and after violent efforts and a further dose of an emetic she vomited altogether about seventy larvae. The

expulsion of the larvae was followed by a regaining of the appetite and sleep. In 1876 Judd described the discharge of the larvae of *F. scalaris* from the intestine of a boy in Kentucky, U.S.A.

Thébault (1901) records the occurrence of dipterous larvae in the intestine with the production of haemorrhage, as is sometimes the case.

Stephens (1905) records the larvae of *F. scalaris* as having been passed *per rectum.* Cattle (1906) refers to a case in which large quantities of the bot-fly occurred in the intestine, there were few symptoms and the larvae were being discharged *per anum* seven months after he first saw the patient. The writer (1909) has also recorded the occurrence of *F. canicularis* in the stools. Recently, Soltau (1910) has recorded the occurrence at Plymouth on the 28th of May of the larva of *F. canicularis* in the stools of a man who had not previously had intestinal pains. The occurrence in September, 1909, in the faeces of a boy aged 12, of the larvae of a species of *Fannia* has been described by Garrood (1910).

Occurrence in the Urinary Tract.

It would appear most unlikely for the larvae of these insects to be discharged from the urinary tract and yet there are a number of records of such occurrences. These have been excellently summarised by Chevril (1909), who found twenty cases of myiasis of the urinary tract recorded in literature. Six of these may be considered authentic, ten as very probable or probable, and four doubtful.

Tulpius (1672) records the passage of 21 small larvae from the urethra. From the figure which is given it would appear that these are *F. canicularis.* In 1792 Veau de Launy recorded the occurrence of and figured a larva which resembles *F. canicularis.* This larva was expelled with the urine by a man of 45. Hagen (1879) gives a summary of the literature up to that date in which twenty cases of insects of various orders are recorded as being expelled with the urine. Eleven of these insects were Diptera. Lallier (1897) gives eleven cases of insects expelled from the urinary tract; three of these cases were not recorded by Hagen. Chevril (*l. c.*), in addition to a critical examination of the previous records, as already mentioned, gives an additional case of the occurrence of the larva of *F. canicularis* in a woman of 55 who suffered from albuminuria and urinated with much difficulty. On May 26th, thirty to forty larvae of *F. canicularis* of different sizes were passed.

Mode of infection.

The larvae of the flies belonging to the genus *Fannia* inhabit, as will be seen from the preceding account of their breeding habits, excrement and decaying vegetable products; and the female flies, guided by their sense of smell and impelled by their maternal instincts, seek such substances. They are attracted to excrement, decaying, putrefying or purulent substances. These facts render several methods of infection possible.

In the case of intestinal myiasis, the flies may have deposited their eggs in or upon rotting or decaying fruit, vegetables or other food which may be eaten in a raw state, and thus the eggs or young larvae will be taken into the digestive tract. Or, the flies, which are generally to be found depositing their eggs in the old-style privies, may deposit their eggs in or near the anus, especially if the person is somewhat costive. The larvae, on hatching, make their way into the rectum and thence into the intestine. This latter mode of infection is probably the common one in the case of infants belonging to careless mothers. Such infants are sometimes left about in an exposed and not very clean condition, in consequence of which flies are readily attracted to them and deposit their eggs.

The infection of the urinary tract is more difficult to understand. The flies are no doubt attracted to the genital apertures by the different albuminous secretions, spermatic, menstrual, gonorrhoeal or leucorrhoeal. The larvae would feed upon the muco-purulent secretions. It is easier to understand the infection of the urinary tract of a woman rather than that of a man. The case recorded by Chevril indicates fairly clearly how the female urinary tract may be infected by the continued or prolonged exposure of the organ. As the flies are frequently found in bedrooms the infection of both sexes during hot weather is sometimes rendered possible.

The whole subject of the relation of these flies to myiasis of the intestinal and urinary tracts is one which has received comparatively little attention. Certainly not the attention it deserves on account of the complications incident to such infections that may arise.

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PROTISTS PARASITIC IN THE LARVA OF THE CRANE-FLY, *TIPULA* SP.

(Preliminary Note.)

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(With 27 Text-figures[1].)

Introduction.

In 1892 Louis Léger described three species of gregarine from the alimentary tract of the larva of *Tipula*: *Actinocephalus tipulae*, *Clepsidrina longa*, and *Eirmocystis ventricosa*[2].

The object of the present note is to record the presence of certain other protists abundant in the same host, but apparently unnoticed hitherto. Several of these offer points of special interest, the details of which I hope to publish soon.

All the larvae examined by me came from the neighbourhood of Aberdeen, and I have found them almost all parasitized to some extent[3]. While engaged on this piece of work, I heard from M. Alexeieff (Paris) that he had observed similar protozoa in *Tipula* larvae from Grenoble and the neighbourhood of Paris. Alexeieff's material was not nearly so richly infected as mine, but I have had the opportunity of seeing his preparations, and find that they contain all the forms observed by me in the larvae from Aberdeen. I should like to acknowledge here my great indebtedness to M. Alexeieff for his generosity in placing his material at my disposal, and in giving me the benefit of his large experience among the flagellate protozoa.

[1] All figures drawn to scale under Zeiss comp. oc. 12 and apochromatic obj. 2 mm.

[2] *C. longa* has since been altered to *Gregarina longa* and the genus *Eirmocystis* corrected to *Hirmocystis* (vide *Das Thierreich. Sporozoa.* Labbé, 1899).

[3] During the summer of 1911, I examined a large number of the adult flies, but never found any trace of infection.

Description of Parasites.

I. Rhizopoda.

The Rhizopoda are represented in the gut of *Tipula* by two small forms of *Entamoeba*.

1. One of these (Figs. 1 and 2) has a nucleus of the *Amoeba limax* type, with well-developed central caryosome and peripheral grains of chromatin contained within a very delicate nuclear membrane.

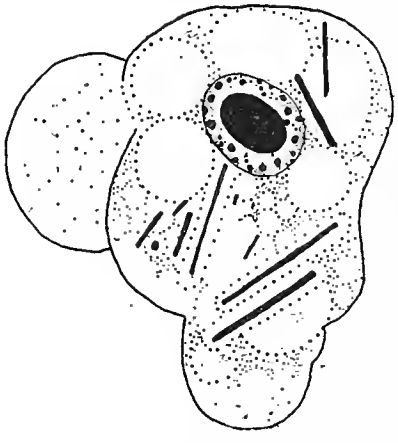

Fig. 1.

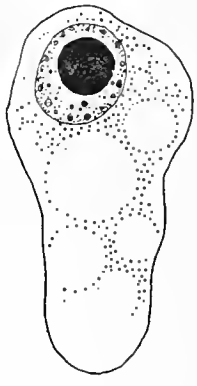

Fig. 2.

Fig. 1. Large *Entamoeba* with nucleus of *Amoeba limax* type.
Fig. 2. Small *Entamoeba* of same type as in Fig. 1.

2. The other (Figs. 3 and 4) resembles the entamoebae of vertebrates[1] in that its nucleus has the chromatin concentrated in an irregular layer immediately internal to the well-marked nuclear membrane, while the interior contains typically one small central chromatin granule. At times the chromatin is collected into two or three large caryosome-like masses placed excentrically (Fig. 5): such nuclei resemble closely the figures recently given by Hartmann for degenerate *Entamoeba tetragena*.

Both forms of amoeba have a coarsely vacuolated cytoplasm containing numerous inclusions, notably bacilli. There are usually several short, blunt pseudopodia, in the formation of which there sometimes appears a distinct severance of clearer ectoplasm from more granular endoplasm.

[1] Fantham and Porter (1911) have recently recorded an entamoeba from the hive-bee (*Entamoeba apis*) "very like *Entamoeba coli* of the human intestine."

The amoebae of the second type are sometimes parasitized by a micrococcus (cf. Nägler, 1910), accompanied by nuclear hypertrophy and other signs of degeneration.

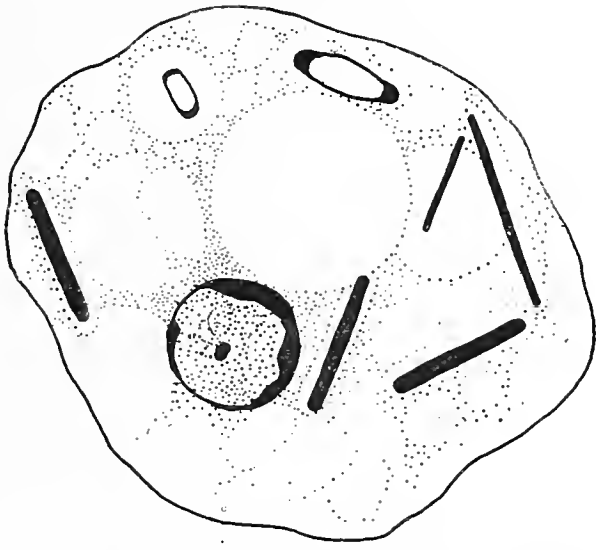

Fig. 3. Large *Entamoeba* with nucleus of the type found in the entamoebae of vertebrates.

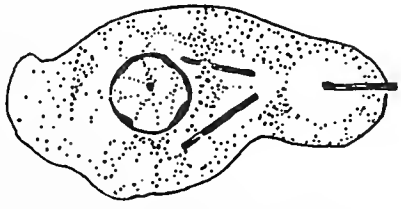

Fig. 4. *Entamoeba* from *Tipula*. Small individual of type 2.

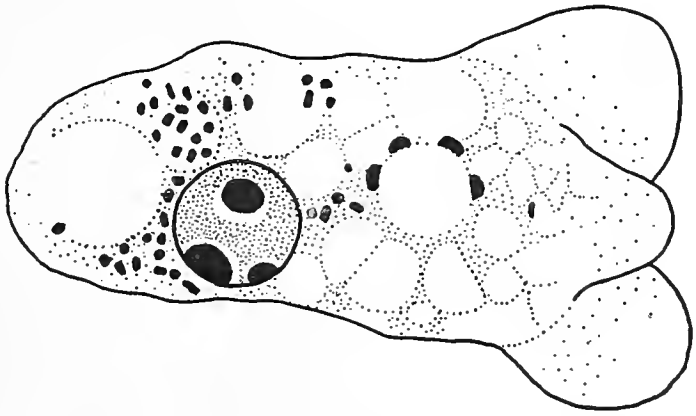

Fig. 5. Large *Entamoeba* of 2nd type. Here the chromatin is disposed in three excentric masses. There are numerous micrococci in the cytoplasm.

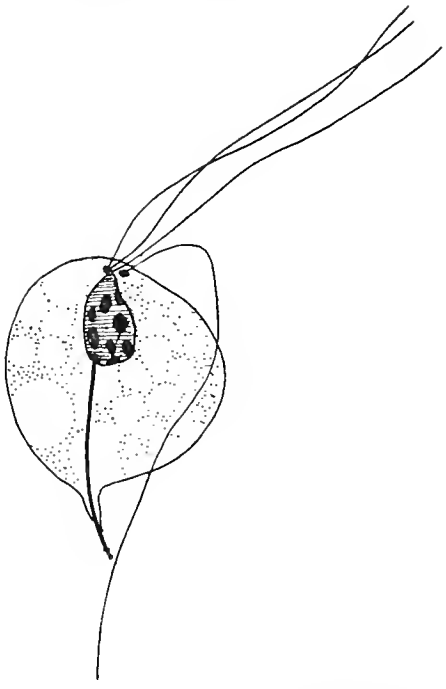

Fig. 6 Flagellate individual of *Trichomastix* from *Tipula*.

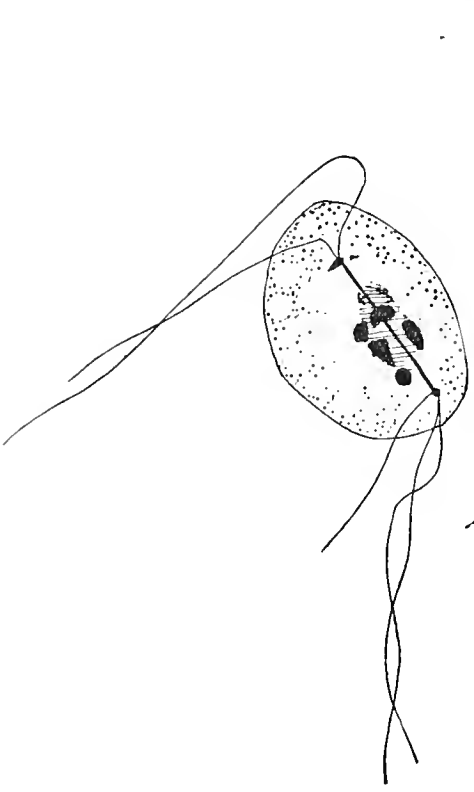

Fig. 7.

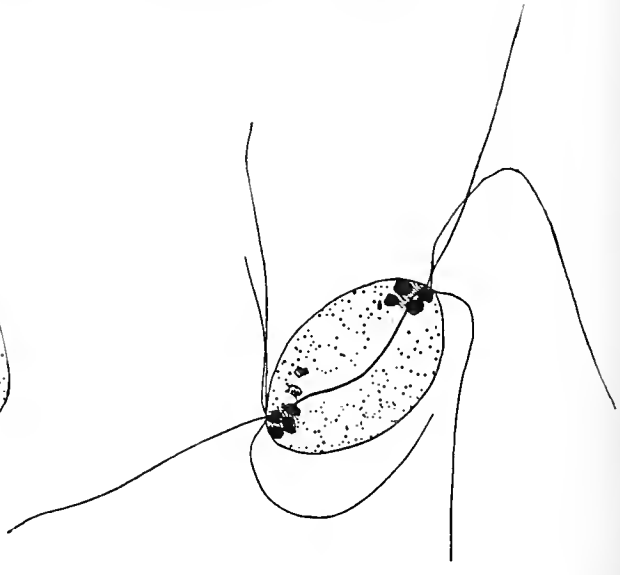

Fig. 8.

Figs. 7 and 8. Stages in division of *Trichomastix*.

There is great variability in size. The average dimensions of the entamoeba of Type 1 are $8\,\mu \times 5\,\mu$ with a nuclear diameter of $3\,\mu$. Individuals are met measuring as much as $16\,\mu \times 15\,\mu$, while others are as small as $5\,\mu \times 5\,\mu$. Type 2 is usually rather larger than Type 1. $16\,\mu \times 17\,\mu$ is a common size, with nuclear diameter of $4\,\mu$. Some are as large as $25\,\mu \times 22\,\mu$: others as small as $8\,\mu \times 5\,\mu$, or even less.

The details of division and encystment have not been worked out, and it is therefore not possible to tell yet whether there are here two different amoebae, or only two aspects of the same species. With more material at my disposal, I hope soon to settle this point.

II. Flagellata.

I find no less than six different flagellates in *Tipula*, viz. a *Trichomastix*, a *Monocercomonas*, a *Polymastix*, a *Hexamitus*, and two species of *Embadomonas*.

1. *Trichomastix.* This flagellate resembles in all important respects the trichomastix described by me from the larvae of Trichoptera. In all the stages that I have found hitherto—the ordinary flagellate individuals ($8\,\mu \times 4\,\mu$), dividing forms, and multiplication (?) cysts

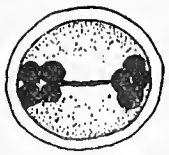

Fig. 9. Cyst of *Trichomastix* from *Tipula*.

($5\,\mu \times 4\,\mu$) (Figs. 6, 7, 8 and 9)—this flagellate, except for its rather smaller size, is morphologically indistinguishable from *T. trichopterorum* Mackinnon. In the dividing flagellates and in the cysts, the chromatin shows a marked tendency to group itself into four masses (chromosomes ?) at each pole.

It occurs in about 30 % of the infected larvae.

2. *Monocercomonas.* This is a pear-shaped or spherical organism without definite periplast (Figs. 10 and 11). Its dimensions vary from $6\,\mu \times 3\,\mu$ to $9\,\mu \times 5\,\mu$. The four *flagella*, which appear to be of approximately equal length, are disposed in two groups of two at the anterior end of the body, the groups usually being separated from one another by a distinct space. The *nucleus*, which lies at the anterior end of the organism, is of the "vesicular" type, with a well-marked caryosome,

sometimes placed excentrically, and also a few chromatin grains in the surrounding nuclear sap. There is generally a distinct group of extra-nuclear siderophilous granules.

The flagella can be seen to take their origin in two *basal granules*, which are sometimes connected by a dark-staining delicate line (Fig. 11). An *axostyle* is usually developed, and appears to arise from one of the basal granules. It then curves around one side of the nucleus, and runs as a slightly sinuous line to the posterior end of the body, beyond which it seldom projects. (Cf. the more rigid axostyle of trichomonads.) The vacuolated cytoplasm contains numerous inclusions, sometimes of astonishing size, considering that there is no hint of a cytostome. The flagellate is sometimes parasitized by a micrococcus.

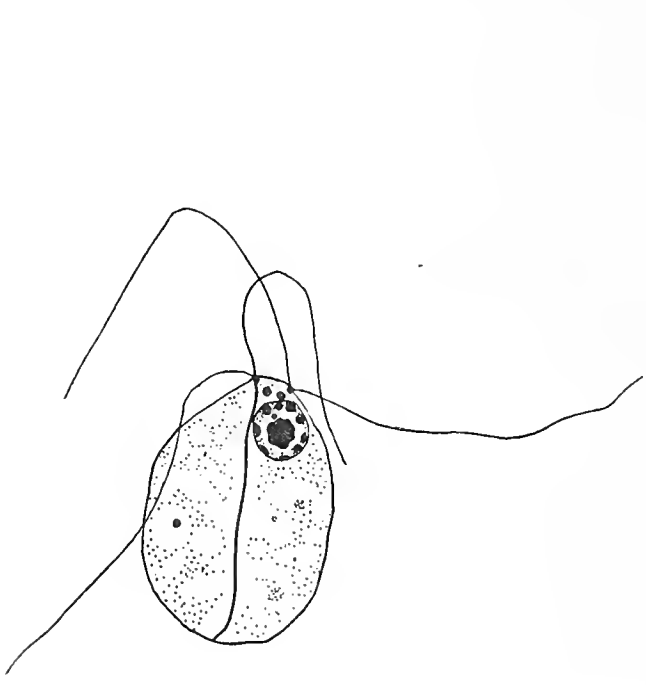

Fig. 10.

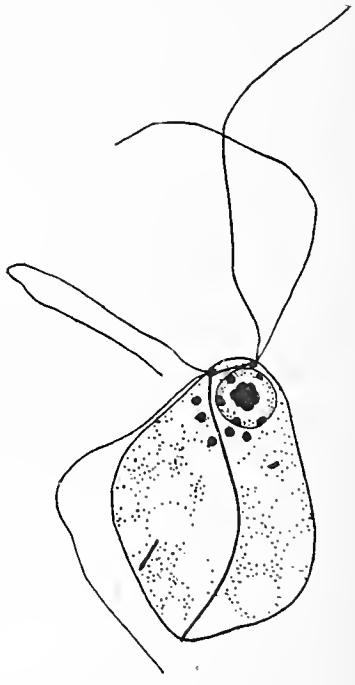

Fig. 11.

Fig. 10. *Monocercomonas* from *Tipula*. Flagellate individual.
Fig. 11. *Monocercomonas*. Flagellate showing connection between basal granules.

The *Monocercomonas* from *Tipula* agrees in all essentials with the closely related species *M. melolonthae* (Grassi) (1879) from *Melolontha* and *Gryllotalpa*, and *M. cetoniae* Jollos (1911)[1].

[1] *M. bufonis* Dobell (1908), the only other well-authenticated species in this genus, differs markedly from these in having no axostyle, and in possessing a well-developed "pseudo-chromidial body." (Alexeieff, 1911.)

In *M. cetoniae* the "outer nuclear network" is "poor in chromatin" (Jollos), and Hamburger finds that the flagella of this species show a tendency to inequality in length, and the development of a "Schlepp-geissel." *M. melolonthae*, on the other hand, is described as having a "wohlentwickelten chromatinreicheren Aussenkern" (Jollos), and, according to Grassi, the flagella are of approximately equal length. So far as I can judge from the scanty indications in the literature, the flagellate from *Tipula* may be placed, at least provisionally, in Grassi's species, *M. melolonthae*, from which it differs only in its somewhat smaller size and the frequent presence of extra-nuclear siderophilous granules.

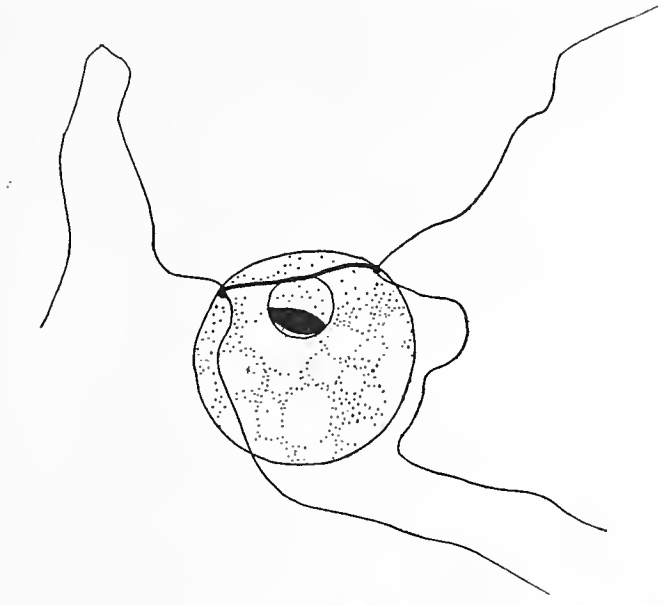

Fig. 12. Division stage of *Monocercomonas* from *Tipula*.

The *division* stages (Fig. 12), so far as I have studied them, agree on the whole with Hamburger's account for *M. cetoniae*.

This flagellate occurs in about 75 % of the infected larvae. I have frequently observed what I consider to be its cysts.

3. *Polymastix*. It is interesting to find that, as in *Melolontha* (according to Grassi (1882) and Hamburger (1911)), and in *Cetonia* (according to Hamburger (1911)), the *Monocercomonas* of *Tipula* is usually accompanied by a *Polymastix*.

This organism, which occurs sometimes in vast numbers, is a pear-shaped flagellate often forked or otherwise "deformed" posteriorly. The dimensions vary from 7 $\mu \times 4\ \mu$ to 15 $\mu \times 6{\cdot}5\ \mu$ (Figs. 13 and 14). Four long *flagella*, approximately equal in length, spring from the anterior

end in two groups of two. The *nucleus* is of the same vesicular form as in *Monocercomonas*, containing a caryosome composed of a number of closely-massed chromatin granules, but with very little chromatin in the space between caryosome and nuclear membrane. As in the *Monocercomonas* the nucleus is surmounted, and in part surrounded, by a group of siderophilous granules. In well-stained individuals it is

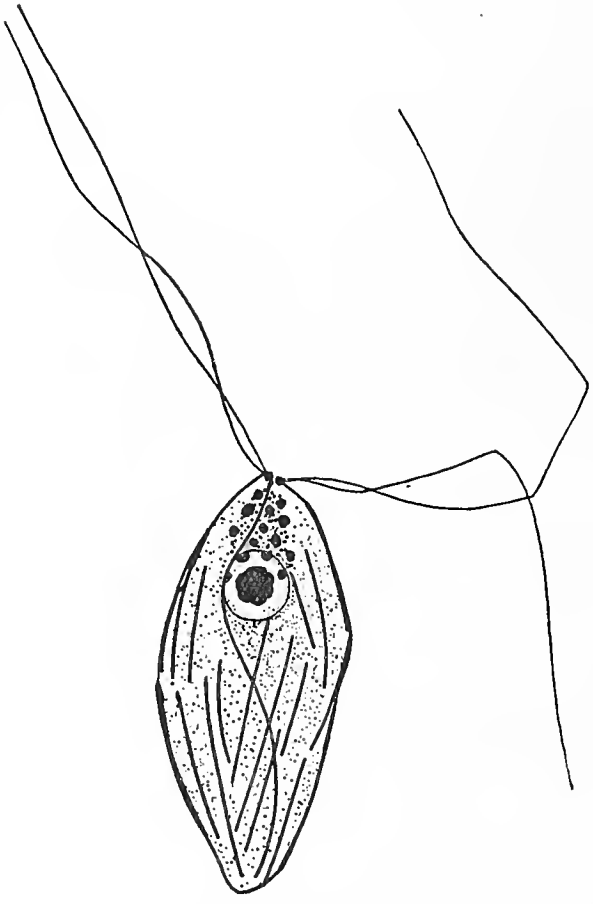

Fig. 13.

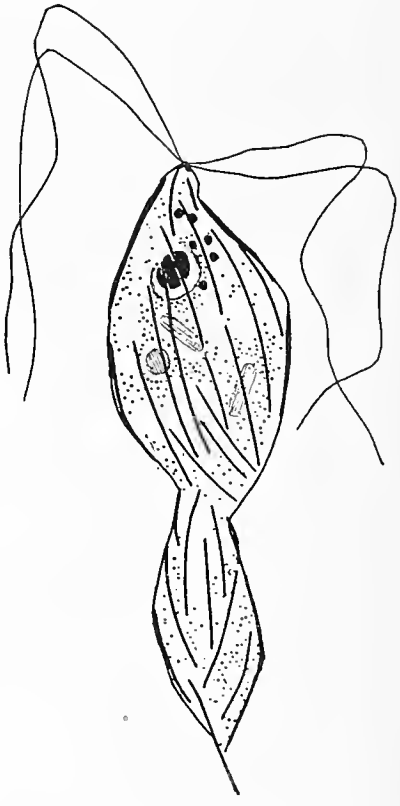

Fig. 14.

Fig. 13. *Polymastix* from *Tipula*. Large flagellate individual showing vesicular nucleus, extra-nuclear granules, axostyle, etc.

Fig. 14. *Polymastix* showing a characteristic posterior deformity.

generally possible to make out an axostyle of the same sort as in *Monocercomonas*, and bearing the same relation to the nucleus and basal granules[1]. In all these points, *Polymastix* and *Monocercomonas* resemble one another closely. The chief feature that distinguishes the

[1] In *Polymastix* the nucleus is usually placed further from the anterior end than in *Monocercomonas*.

flagellate individuals from one another is the periplast, which in *Polymastix* is well-developed, and characteristically raised up into longitudinal "ribs" or minute folds.

These "ribs," which in the living organism appear to run almost unbroken from one end of the flagellate to the other, are seen on staining to be discontinuous, and to fall into groups of shorter, darkly-staining lines inclined at various angles[1]. There appears to be a small cytostome between the two groups of flagella, and the cytoplasm contains ingested bacteria, etc.

In certain cases this periplast disintegrates (Figs. 15 and 16), and it seems to me not impossible that the *Polymastix* may, through loss of its pellicle, pass into a *Monocercomonas* form: in such cases, the two genera, as represented by the parasites from *Tipula* at least, could no longer be regarded as autonomous. A careful study of the division and encystment can alone decide whether this is the case or not. With regard to the division I can as yet say very little, but it appears to me that the axis of the division-spindle comes to lie parallel to the long axis of the body, in which case authors' statements that the division is transverse would not be far out.

The genus *Polymastix* was formed by Bütschli to include Grassi's *Trichomonas melolonthae*. So far, no reliable species have been added to the genus[2], the solitary occupant of which, not examined since 1884, "bedarf dringend der Nachuntersuchung" (Doflein, 1911). Hamburger (1911) has recently published a preliminary note, including observations on *Polymastix melolonthae*. She finds the same parasite also in the larvae of *Cetonia*. In various points, such as the presence of an axostyle and the details of nuclear structure, my flagellate differs from the

[1] Grassi interpreted these structures as "trichocysts." On first seeing them, I was inclined to regard them as bacilli adherent to the body of the flagellate (cf. Künstler, 1882), or else ingested and in some way come to form a sort of thin layer just below the periplast. Various points plead in favour of this view, notably the occurrence of such individuals as that figured in Figs. 15 and 16. However, I have recently abandoned the idea, and consider that Frl. Hamburger's interpretation is probably the right one—"mir scheinen es verdickte Streifen der Pellicula zu sein."

M. Alexeieff has drawn my attention to a similar appearance presented by the periplast of *Lophomonas striata* Bütschli, from the cockroach, recently described in detail by Janicki (1910).

It is interesting that an unstriated form, *Lophomonas blattarum*, lives alongside this. I believe that a comparison of *Polymastix* with these Trichonymphids may not be unprofitable.

[2] The flagellate with six flagella and without striated periplast, placed provisionally by Alexeieff (1911) in this genus as *P. bufonis*, seems to me a very doubtful species.

account given by Frl. Hamburger. But in iron haematoxylin preparations where the differentiation is unsatisfactory, I get much the same homogeneous nucleus as she describes, and can see nothing of the axostyle. I am inclined to regard the *Polymastix* from *Tipula* as identical with the type-species *Polymastix melolonthae* (Grassi).

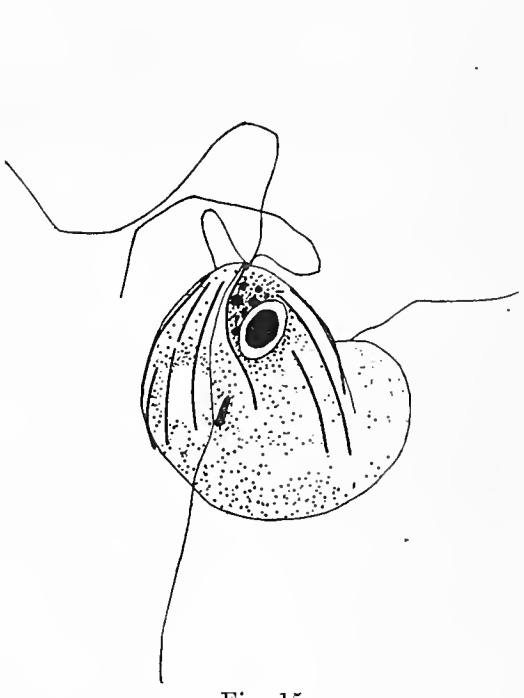

Fig. 15.

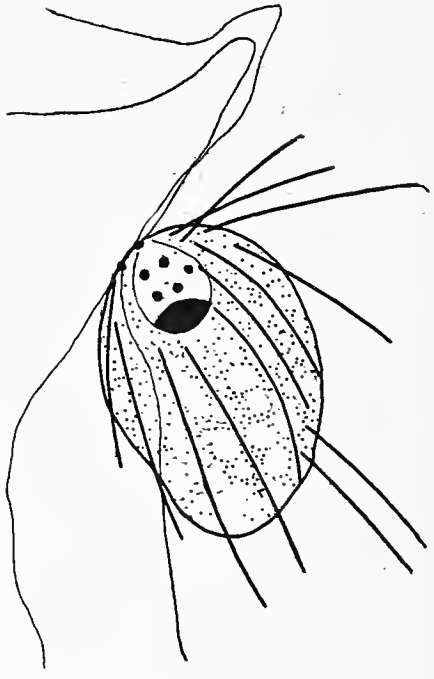

Fig. 16.

Fig. 15. *Polymastix* in which the hinder end has lost its characteristic ribbed pellicle.
Fig. 16. *Polymastix* in which the periplast is fraying off. Possibly some of these "ribs" may in this case be adherent bacteria.

4. *Hexamitus.* In some 10 % of these infected larvae there occurs very sparingly[1] a flagellate which is apparently *Hexamitus intestinalis*, or some very closely allied species (Fig. 17). It is of small size, $8\,\mu \times 3\,\mu$ being the maximum dimensions. Its mode of occurrence suggests that it is more or less a chance parasite, and that *Tipula* is not its true host. *Hexamitus intestinalis* has been recorded from such diverse animals as frogs, toads, tortoises, lizards and fish. It is probably one of those "facultative" parasites, which are not very fastidious as to host, and which may therefore find opportunity to develop in a great variety of situations.

[1] M. Alexeieff informs me that he found it abundant on one occasion in a larva from the neighbourhood of Paris.

5. *Embadomonas.* This genus, formed by me for a monoflagellate slipper-shaped organism in the larvae of Trichoptera, finds two representatives in *Tipula.*

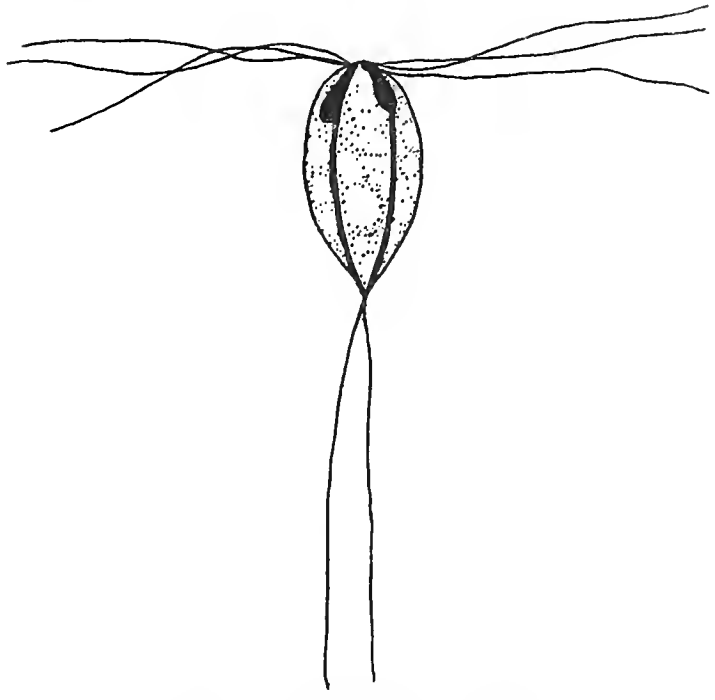

Fig. 17. *Hexamitus* from *Tipula.*

Fig. 18. Fig. 19 *a.* Fig. 19 *b.*

Fig. 18. *Embadomonas agilis.*
Fig. 19 *a.* *E. agilis.* Small individual.
Fig. 19 *b.* *E. agilis.* Individual with vesicular nucleus.

(*a*) The first of these is a small flagellate (4 $\mu \times 1{\cdot}5\ \mu$ to 11 $\mu \times 3\ \mu$) indistinguishable from *E. agilis* Mackinnon from Trichoptera except by its rather smaller size. It has the characteristic slender slipper form, large cytostome, and anteriorly placed nucleus composed of a

group of chromatin granules (Figs. 18 and 19 *a*). As a rule it is possible to make out one flagellum arising from a granule on the anterior border of the cytostome. Very rarely the nucleus is of vesicular form, with a definite nuclear membrane and a central group of chromatin grains (Fig. 19 *b*).

The *cysts* (Fig. 20), now found for the first time, are minute oval bodies (3·5 $\mu \times 3\,\mu$ to $4\,\mu \times 3\,\mu$), enclosed by a double membrane, and containing a group of chromatin granules near the apex and a dark hook-shaped line in the position of the cytostome border.

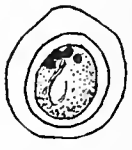

Fig. 20. Cyst of *Embadomonas agilis*.

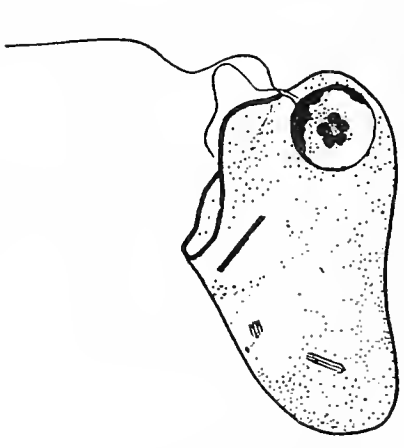

Fig. 21 *a*.

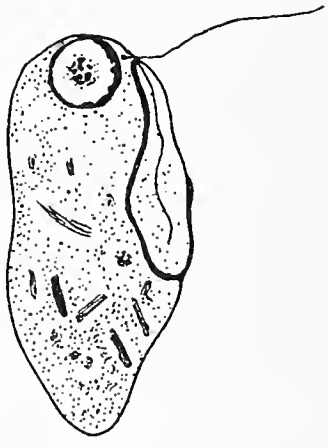

Fig. 21 *b*.

Fig. 21 *a*. *Embadomonas alexeieffi*.
Fig. 21 *b*. *Embadomonas alexeieffi*. Slender individual.

(*b*) *Embadomonas alexeieffi* n. sp. This is a larger flagellate ($12\,\mu \times 8\,\mu$ is a common size, while large individuals measure as much as $16\,\mu \times 7\,\mu$), of clumsier form, and with a much thicker periplast (Figs. 21 *a* and *b* and 22). The *cytostome* is not quite so large in proportion as in the foregoing species, makes an angle with the long axis of the body, and has a very much more definite, deeply-staining border, often thrown into folds. The *nucleus* is almost always vesicular, with a definite membrane, and a central group of chromatin granules on a faint network. A deeply-staining rod-shaped or crescent-shaped mass lies against the nuclear membrane: in some cases it appears to be

extra-nuclear. I have observed two *flagella*, one of which is locomotor in function, while the other generally lies within the cytostome and aids in the ingestion of food-particles. Each flagellum arises from a distinct basal granule near the anterior border of the cytostome. The highly vacuolar cytoplasm is often full of ingested bacteria. The *cysts* are of the same form as those of *E. agilis*, but are proportionately

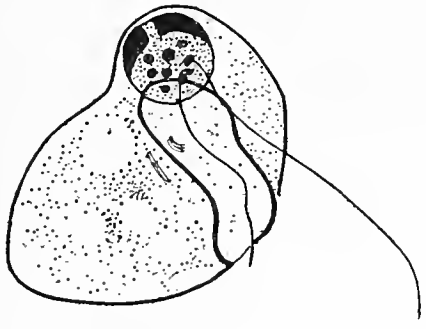

Fig. 22.

Fig. 23.

Fig. 22. *Embadomonas alexeieffi*. Individual with double crescent-shaped chromatin mass. Early stage of division?

Fig. 23. *Embadomonas alexeieffi* containing numerous ingested bacilli.

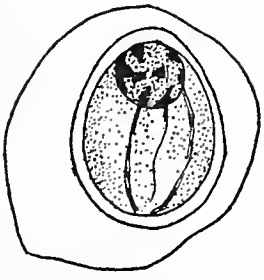

Fig. 24.

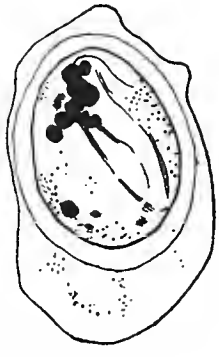

Fig. 25.

Fig. 24. *E. alexeieffi*. Early stage of encystment.

Fig. 25. *E. alexeieffi*. Later stage of encystment.

larger ($5\,\mu \times 4\,\mu$ to $6\,\mu \times 5\,\mu$) (Figs. 24 and 25). In the newly-formed cyst the nucleus is of the vesicular type, but it soon loses its membrane, and the chromatin escapes to form irregular groups of granules in the anterior end of the cyst. The borders of the cytostome persist as dark-staining, loop-shaped strands. There is frequently visible around the

young cyst a delicate envelope also of oval form, within which the cyst lies excentrically. This is the periplast of the flagellate, within which the cytoplasm has contracted, surrounding itself with the true cyst membrane. This outer envelope disintegrates and disappears from the older cysts. Fig. 26 shows small oval flagellates (3·5 $\mu \times 2{\cdot}5\ \mu$) probably just emerged from the cysts. I have never seen more than one flagellum in these small forms.

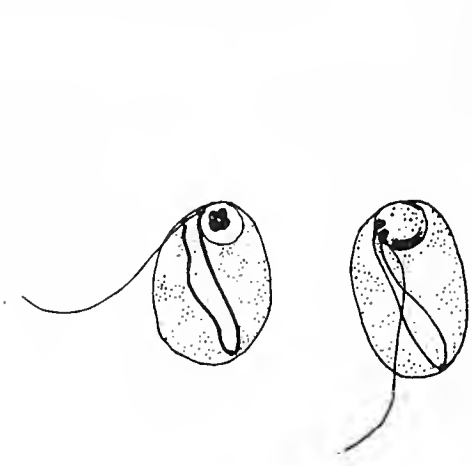

Fig. 26.

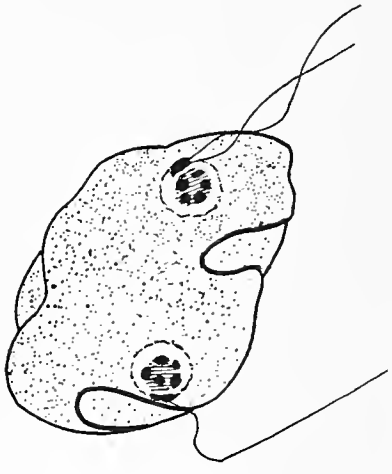

Fig. 27.

Fig. 26. *E. alexeieffi.* Small individuals.
Fig. 27. *E. alexeieffi.* Stage in division.

I have observed a few division-stages of *E. alexeieffi* (Fig. 27), which agree with the figures of Robertson and Martin (1911) for the division of *Chilomastix gallinarum.*

I have recently had the opportunity of making a comparison between the embadomonads of *Tipula* and the species of *Chilomastix* described by Alexeieff. I am now persuaded of the close relationship between the two genera, to which *Fanapepea* Prowazek (1911) is probably also related[1].

III. Bacteria.

Bacteria occur in enormous quantities. Chief among them is a large sinuous form resembling *Bacillus flexilis* Dobell.

[1] A further diagnosis of the genus *Embadomonas* is necessitated by the details of structure revealed in the large form *E. alexeieffi*: but I prefer to hold this over until I have worked out the life-cycle more fully.

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HAEMOGREGARINA ANARRHICHADIS FROM *ANARRHICHAS LUPUS*, THE CATFISH.

By HERBERT HENRY, M.D.

(*From the Department of Pathology, University of Sheffield.*)

(With Plate VIII.)

The haemogregarine which is the subject of the present communication was obtained in material collected by me during two voyages made in a trawler in July 1910, round the Shetlands and the north coast of Scotland. It was found in large adult specimens of catfish (*Anarrhichas lupus*), taken by otter trawl from a depth of 50 to 80 fathoms, in the vicinity of Fair Isle and Foula, and on Whitenhead Bank about nine miles to the N.N.E. of Cape Wrath. Catfish taken near Rhona and Sulisker in the Atlantic showed no infection, but the number of fish examined was so small that one could not assume the infection to be absent in this locality.

Films were made with blood taken directly from the heart. They were allowed to dry in air, then fixed in absolute methyl alcohol for 30 minutes, and packed in oiled paper to be stained at the laboratory later. The description I am about to give of the parasite applies therefore only to the appearance it presents in these films stained with Leishman or Giemsa. It is of course impossible to microscopically examine fresh blood on board a modern commercial trawler while it is on the fishing grounds, and attempts to obtain wet-fixed films end in disaster. The preparation even of air-dried films is beset with many difficulties and disappointments, which only those can fully realise who have attempted such work in rainy weather and on stormy seas.

Morphology of the parasite.

The haemogregarine was found in six out of sixteen fish examined but in all of these the infection is very slight. It never occurs free in the plasma but is always intracellular. Infected red corpuscles harbour only one haemogregarine, so that multiple infections have never been observed. The nucleus of the host cell is frequently pushed to one side, particularly by the larger forms of the parasite, but no other change in shape, size, or staining reactions of either the nucleus or the protoplasm of the host cell is evident. The parasite is not surrounded by a capsule or cyst. It is met with in the blood in several different phases of development, which for descriptive purposes may be roughly divided into three main types, viz.

1. A short, broad, oval or pear-shaped form.
2. A long, thin form, thickened at one pole and more or less pointed at the other, and slightly bent so as to be comma-shaped.
3. A long, thick, slightly curved type which is reniform.

These types are not to be looked upon as sharply demarcated one from the others. For instance, there are very numerous phases of development between types 1 and 3, or between types 2 and 3. Moreover these types are not to be found in equal proportion throughout the whole series of films. In two of the infected fish type 1 largely preponderates, and in another two, type 3 is predominant. In the remaining two infected fish specimens of type 1 and type 3 are about equally distributed, whereas in all type 2 occurs but rarely.

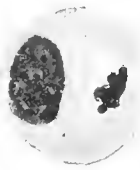
Type 1 (Pl. VIII, figs. 1 to 10.)

The earliest stage consists of small, oval or pyriform bodies varying from 5 μ to 6·8 μ in length, the average being 6 μ, and varying from 3·3 μ to 4 μ in breadth, the average being 3·7 μ. The nucleus, which is always situated at the narrower pole of the parasite, varies from 2 μ to 4 μ in its longest diameter, the average length being 2·8 μ. It does not occupy the whole thickness of the lesser pole, but is usually separated from the margin of the parasite by a thin rim of protoplasm, and appears to consist of a varying number of coarse, deeply staining chromatin granules, which tend to remain loosely bunched together. Occasionally a string of chromatin granules may be found wandering away from the main nucleus into the body of the parasite (fig. 2). The

protoplasm of these oval forms in their earliest stages stains very faintly, so that sometimes the contour of the parasite is discernible only by contrast with the surrounding protoplasm of the host cell. Later, it takes on a distinct pale blue stain, with irregular and ill-defined areas of condensation scattered here and there (figs. 6, 7, 9), and, as the haemogregarine gets older, the colouring of the protoplasm deepens into a dull purple tint (fig. 8), while the more deeply staining patches of condensation become more marked. As a rule the protoplasm shows no granulation. Once only (fig. 4), the broader pole was found to be occupied by a ring of fine granules. Not infrequently this pole is occupied by one or two large clear vacuoles (figs. 6, 10). But much more characteristic is the gradual development in this broader pole of some purple staining substance, at first staining faintly (fig. 5), but soon staining more and more deeply, until it finally appears as a purple black mass, more intensely stained even than the chromatin granules of the nucleus. This substance varies in shape from a thin crescent the horns of which run up the sides of the haemogregarine (figs. 3, 8, 9), to a large semilunar mass which completely fills up the broader pole (figs. 6, 10). This appearance is a still more marked feature of the larger forms of the haemogregarine.

Type 2. (Pl. VIII, figs. 11 to 20.)

This phase is longer and much thinner than the foregoing. The length varies from 8·5 μ to 9 μ, with an average of 8·7 μ. The breadth ranges from 1·5 μ to 3 μ, the average being 2·5 μ. One extremity is usually slightly more pointed than the other, and the parasite as a whole is bent so as to be comma-shaped. The nucleus, which lies in the pointed extremity, averages 3·2 μ in length, and thus takes up more than a third of the total length of the parasite. The nuclear chromatin granules are considerably smaller and do not stain quite so deeply as in the case of type 1. The protoplasm, particularly in the thinner forms, takes on a dull purple tint (figs. 11 to 15). The clubbed extremity of the haemogregarine is filled with the deeply staining substance before mentioned, which may take up a quarter or even a third of the total length of the parasite. This substance frequently ends abruptly on the nuclear side, being sharply demarcated, by a straight line, from the rest of the parasite. The vacuoles occurring in the later phases of type 1 are here but rarely seen, and when seen are often indistinct in outline.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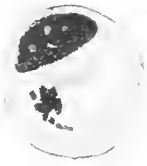
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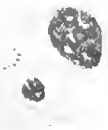
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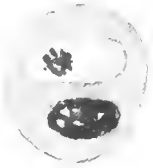
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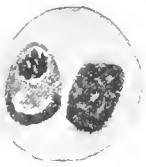
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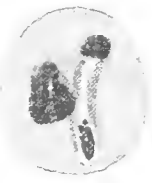
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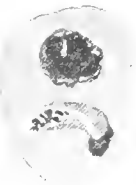
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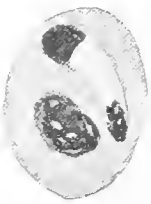
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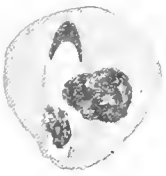
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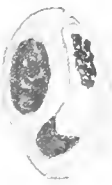
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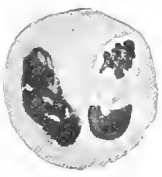
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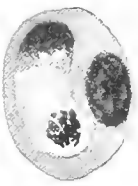
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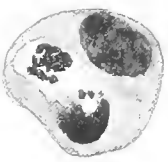
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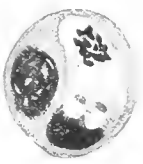
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Type 3. (Pl. VIII, figs. 21 to 30.)

These are 8 μ to 9·4 μ long, the average length being 8·7 μ, and 3·5 μ to 4 μ wide, the average width being 3·6 μ. Most of these large broad forms would appear to be adult specimens of many of the forms in type 1. It is possible too that some of them may result from the long thin forms by a simple increase in width. The protoplasm is paler than that in type 2, with Leishman and Giemsa staining being more distinctly pale blue, and with none of the purple tint so frequently found in the latter. The nucleus is larger, but relatively not more so than that in type 2, when one takes into consideration the increase in bulk of the whole parasite. The nuclear chromatin is more coarsely granular, and at times takes on the appearance of deeply staining rods, which zigzag across the parasite transversely (fig. 27). The pole opposite the nucleus is still occupied by the deeply staining, purplish black material found in other forms; but, on its inner aspect this substance does not end so abruptly as in specimens of type 2, and very frequently there lie actually in or in close proximity to this inner margin one or two clear round vacuoles (figs. 23, 27—30). Also there are to be found sometimes on the nuclear side of these vacuoles one or two deeply staining granules (figs. 27, 29). It is impossible to decide whether these are chromatin elements or whether they represent small pieces of material which have become separated from the main polar mass.

Comparison with known forms.

This haemogregarine of the catfish is quite distinct from the more commonly occurring haemogregarines met with in seafish. It differs markedly from the schizohaemogregarines, viz. *Haemogregarina bigemina* of the common shanny, *Blennius pholis* (Laveran and Mesnil, 1901), *Haemogregarina quadrigemina* of the dragonet, *Callionymus lyra* (Brumpt and Lebailly, 1904) and *Haemogregarina simondi* of the ordinary sole (*Solea vulgaris*, Laveran and Mesnil, 1901) for it shows no evidence of intracorpuscular fission. Moreover, the nucleus throughout is larger, being composed of bigger granules, and stains much more deeply than do those of the schizohaemogregarines treated under similar conditions as regards fixation and staining. Some of the long thin forms, and of the forms intermediate between types 2 and 3, resemble in size and contour the haemogregarine (Lebailly, 1904) met with in *Pleuronectes platessa*, and that occurring in *Cottus bubalis* (Brumpt and Lebailly, 1904), but in these latter the nucleus is more

centrally situated, occupies the whole width of the parasite so that it often bulges laterally, and readily overstains with Giemsa or Leishman, so that it is impossible, without extraction, to make out the chromatin elements distinctly. Moreover, *Haemogregarina platessae* frequently exhibits at one pole a clear vacuole, which is quite absent from the catfish haemogregarine.

The existence of the phases above described, the occurrence of the deeply staining polar mass, and the features of the nucleus in the latter distinguish it from all previously described piscine haemogregarines with the exception of *Haemogregarina rovignensis*, a parasite found by Minchin and Woodcock (1910) in three specimens of a gurnard, *Trigla lineata*, caught at Rovigno on the Adriatic. In both there occur the same three phases of development, and the small oval forms are closely alike in each case. But in the case of the long thin forms in the gurnard haemogregarine their pointed extremities are frequently folded back on the main body of the parasite, whereas this appearance has never been found in the catfish parasite. It is not improbable however that this form does exist, but is obscured in my films from the method of preparation employed, for in working with a snake haemogregarine I have been struck with the paucity of forms showing the recurved limb in air-dried films as compared with the relative frequency of such forms in wet-fixed preparations. Again, in *Haemogregarina rovignensis* the deeply staining polar mass is not so constant as it is in the catfish haemogregarine. In the case of the former, too, there is a marked difference between the long thin form with a compact nucleus and the long thick bean-shaped form with a relatively larger nucleus and transitional phases are absent. On the other hand, a striking feature in films of the catfish haemogregarine has been the rarity of long thin forms and the frequency of stages intermediate between these thin forms and the thick reniform type. Also the nucleus of the thick forms does not appear to be relatively larger than that of the thin type. Such distinctions, of course, may be apparent rather than real, for in the case of the gurnard parasite the description is taken from wet-fixed films, whereas the picture presented by the catfish haemogregarine is merely that shown in films fixed after being dried in air.

Lastly, there confronts one the interpretation of the significance of the various phases of the parasite found in the circulating blood. At the outset one may state that the different forms represent phases in the development of one and the same haemogregarine, for there is no reason for assuming that they represent different infections, *i.e.*

infection with three distinct parasites. The young oval forms of *Haemogregarina rovignensis* are described as being derived from sporozoites which have penetrated the red blood corpuscles and which are destined to become schizonts. It is much more likely that the sporozoites penetrate the cells of some of the internal organs and that the oval forms found in the red corpuscles are the resulting merozoites. It has also been suggested that the large forms of the gurnard haemogregarine show sexual differentiation, the long thin forms with a small nucleus being of male character, and the broad bean-shaped forms with a large nucleus being of female sex. Also, the large forms are taken to represent an old infection, while the small oval forms are taken to represent a new or recent infection. Such an interpretation is merely a matter of conjecture, for we have no knowledge of the time necessary for the full development of a piscine haemogregarine either in the tissues or in the red corpuscles.

If the large forms do show sexual differentiation, then one must assume the existence of a cycle such as occurs in the Haemosporidia, in which sexual features appear only in the last generation of a schizogony where normally no sexual dimorphism occurs; or, one must assume that there occurs a cycle such as has been found in some of the coccidia, a cycle in which micro- and macroschizonts give rise to a crop of micro- and macromerozoites. Nor is it possible to pronounce definitely in favour of either of these assumptions—although the first is probably the correct one—till the opportunity presents itself not only of examining the tissues of the vertebrate host but also of tracing the phases of development in an invertebrate carrier.

On the gills of all the catfish examined there occurred a leech which was sometimes present in enormous numbers. This leech has not been definitely identified, but from a superficial examination of several specimens Miss Muriel Robertson was kind enough to suggest that it is probably *Trachelobdella lubrica*. Several examples of this leech have been examined in carefully prepared serial sections, but no developmental phases have been found, though it is likely that it is the actual carrier of the infection, for a leech, *Ozobranchus Shipleyi*, has been proved to be the carrier of a haemogregarine infection in the case of the common lake tortoise of Ceylon, *Nicoria trijuga* (Robertson, 1910). One must not, however, overlook the possibility of a haemogregarine infection in fish occurring through the alimentary tract from ingestion of an intermediate host, as is described by Miller (1909) for *Hepatozoon perniciosum*.

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ON TWO NEW TREMATODE PARASITES FROM BRITISH FOOD-FISHES.

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(*Lister Institute of Preventive Medicine, London.*)

(With Plate IX.)

In September, 1908, an opportunity was afforded me of examining some of the larger food-fishes brought in to Aberdeen fish market. For this I have to thank the courtesy of Professor J. Arthur Thomson, who kindly provided the necessary laboratory accommodation, and was of great assistance to me in various other directions. I have also to thank the Government Grant Committee of the Royal Society for defraying the expenses of procuring specimens.

I was enabled to obtain several species of fish which I had not met with during my earlier investigations at St Andrews. Amongst these were a few specimens of the horse-mackerel (*Trachurus trachurus*) and the sea-bream (*Sparus centrodontus*), each of which furnished a new and interesting species of parasite. During the short time at my disposal only a couple of horse-mackerel and a dozen bream were obtained and the number of Trematode parasites they contained was very small. A larger number of these fishes have since been collected in various localities, chiefly at Plymouth, but they have added only sparingly to the number of parasites. The form in the horse-mackerel has indeed not been met with again; that from the bream has been found on seven other occasions. Both forms present several interesting peculiarities, and they are not only new species but are types of new genera. For the parasite from the horse-mackerel I propose the name, *Ancylocoelium typicum*, n.g., n.sp., and for that from the bream, *Zoonogenus vividus*, n.g., n.sp.

Ancylocoelium typicum, n.g., n.sp. (Pl. IX, fig. 1).

This is a moderately small Trematode measuring 2·25 mm. in length and 0·48 mm. in maximum breadth. It is decidedly flattened dorso-ventrally and its outline is somewhat spatulate, the greatest breadth occurring in front of the middle of the body, whence it tapers sharply to the anterior end and more gradually to the posterior end. The average breadth in the posterior half of the body is 0·28 mm. In life the animal has a rich yellow colour from the enormous number of eggs which it contains. The cuticle is beset for the greater part of its extent with small scaly spines, which disappear gradually towards the posterior end.

The suckers are small and inconspicuous. The subterminal oral sucker has a diameter of 0·10 mm., while the ventral sucker measures ·13 mm. The latter is situated at a distance of 0·92 mm. from the anterior end.

The somewhat elongated pharynx follows almost immediately on the oral sucker and measures 0·08 × 0·043 mm. The oesophagus is long and narrow. Its length is about 0·5 mm. and the bifurcation takes place a short distance in front of the ventral sucker. The intestinal diverticula are of unique configuration. They are considerably wider than the oesophagus and instead of passing round the ventral sucker they are bent back abruptly into the anterior part of the body. They diverge at a fairly wide angle, passing out towards the sides of the body, but when they have nearly reached the margin they are again bent somewhat acutely and pass back almost straight. They terminate a short distance behind the level of the intestinal bifurcation.

Owing to the enormous mass of eggs, only the terminal part of the excretory vesicle could be observed even in the living specimen.

The genital system presents several peculiar features. The anterior testis is situated somewhat to the right side, a short distance behind the ventral sucker, from which it is separated by the ovary. The posterior testis lies more median, not far behind the anterior testis (0·22 mm. behind the ventral sucker). Both are elongated oval and measure about 0·19 × 0·10 mm., their long axes being slightly oblique to the long axis of the body. The cirrus-pouch is of simple type and is situated over and in front of the right anterior quadrant of the ventral sucker. Posteriorly it is almost contiguous with the ovary and the anterior testis. It shows the usual shape and contains a large oval vesicula seminalis. Its dimensions are 0·32 × 0·11 mm. There is a

very short pars prostatica and a comparatively short and straight ductus ejaculatorius, no part of which was exserted. The genital aperture is situated in the middle line between the intestinal bifurcation and the anterior border of the ventral sucker, but decidedly nearer the former.

The ovary is wedged in between the anterior testis, the ventral sucker and the cirrus-pouch. It is about the same size as the sucker (greatest diameter 0·14 mm.) and its outline is almost globular. The presence of a receptaculum seminis or Laurer's canal could not be ascertained owing to the enormous number of eggs in the vicinity of the ovary. The yolk-glands are situated in the sides of the body at the level of the testis. They are slightly asymmetrical, that on the left being a little in advance of the other. The latter extends from the anterior border of the anterior testis to about 0·7 mm. from the posterior end of the body. They appear to have an unusual configuration, the follicles being arranged in tubules or rather in one continuous tubule which is thrown into a rosette-shaped knot; the rays of the rosette, however, are not equal, those directed forward being decidedly longer than those directed backward. On ventral view only the lateral aspect of the rosette is seen. The yolk-ducts were not observed.

The uterus, which fills the remainder of the posterior part of the body, is extremely voluminous and contains a very large number of small eggs. The uterine walls could not be made out distinctly, but it seems certain that the uterus is not convoluted, the enormous number of eggs being accommodated by a dilatation of the uterine tube. This runs from the ovary, on the right side, backwards to the end of the body, where it turns and passes forward on the left, overlapping the descending limb to some extent. It still retains a considerable width even on passing in front of the ventral sucker and it is joined to the small genital sinus by a very short vagina.

From the genital sinus another unusual and peculiar structure arises. This is a pear-shaped pouch with a narrow neck, which lies between the left intestinal diverticulum and the uterus. The latter separates it from the ventral sucker. It is smaller than the cirrus-pouch, about 0·24 × 0·11 mm., and appears to contain no structure or contents. It seems to be simply a thin-walled sac, and no clue is afforded to its function.

The mature eggs are bright yellow in colour, with oval outline and measure on an average 0·024 × 0·013 mm.

This form possesses several remarkable features which distinctly mark it off as a new type. The chief of these are the accessory genital

sac, the unique configuration of the intestinal diverticula and the peculiar condition of the yolk-glands. It displays no close affinity to any hitherto described Trematode but for systematic purposes it may, for the present, be placed in the vicinity of the *Haplocladinae* (Odhner 1911).

Zoonogenus vividus, n.g., n.sp. (Pl. IX, fig. 2).

This is neither such a remarkable, nor such an uncommon, species as the preceding. It was met with in two out of 11 specimens of the sea-bream (*Sparus centrodontus*) examined at Aberdeen (September) and I have since met with it in five out of eight bream examined at Plymouth (July—August). It was absent, however, in half-a-dozen bream obtained from Billingsgate (November). Its frequency, from these figures, is therefore seven out of 25.

The sea-bream does not occur in inshore waters ; it is only obtained at a considerable distance from the shore. For that reason all the specimens examined had been dead for some time, and the parasites were usually in a more or less macerated condition. The species, however, is a particularly delicate one, judging from the fact that in the same fishes such forms as *Derogenes varicus* and *Hemiurus communis* were still alive and active.

This new species occurred invariably in the rectum and in no other part of the intestine. Only two or three specimens were found in each case. The worms were easily picked out by reason of their remarkably vivid, blood-red colour. The rectal contents of the sea-bream, partly from the fact that it feeds largely on Crustacea, are usually of a dull brownish red colour, but the colour of the parasite was much more intensely red.

It is a small worm, obviously belonging to the family Zoogonidae, and bearing much resemblance in structure and habitat to the commoner and more familiar species, *Zoogonoides viviparus*. Its length is on an average 1·4 mm. and its breadth 0·46 mm. It is somewhat oval in outline, not much flattened, and the greatest breadth is rather behind the middle of the body. As already remarked, the colour is uniformly blood-red, but on close examination the coloration is found to be blotchy, much as in *Zoogonoides viviparus*. The cuticle is covered in the anterior half with minute regularly arranged spines, but these disappear towards the posterior end of the body.

The oral sucker is subterminal and measures about 0·16 mm. in diameter. The ventral sucker is much larger, 0·34 mm. in diameter,

and is situated behind the middle of the body (0·9 mm. from the anterior end). The diameter of the ventral sucker is therefore distinctly more than twice that of the oral sucker and in some cases it is nearly three times. Both suckers are globular.

The pharynx is contiguous with the oral sucker and measures 0·07 mm. in diameter. It is succeeded by a short oesophagus, rather shorter than the pharynx. The intestinal diverticula are simple, narrow and short, terminating some distance in front of the ventral sucker.

The excretory vesicle is a short bulbous sac at the posterior end of the body, containing in many cases a hard yellowish green or brown concretion of irregular shape.

The testes are two ovoid bodies situated one over each posterior quadrant of the ventral sucker, their posterior borders being just a little behind the posterior margin of the sucker. Their long axes are oblique and almost tangent to the edge of the sucker. They measure about 0·25 mm. × 0·12 mm. The cirrus-pouch is a conspicuous arcuate structure lying immediately in front of the ventral sucker. In most cases it is entirely in front of the sucker, but in some it touches or even slightly overlaps it. It contains a bi-partite vesicula seminalis, the posterior half being the larger and measuring 0·1 × 0·07 mm., while the anterior half is almost globular and has a diameter of ·065 mm. In front of this is a bulbous pars prostatica which is slightly larger than the anterior part of the vesicula (0·08 mm.). The rest of the cirrus-pouch (·3 mm.) is traversed by a long ductus ejaculatorius, the walls of which are characteristically crenated or folded. No spines are present in the ductus or cirrus. The genital aperture is situated over the left intestinal diverticulum near its termination. It is therefore not absolutely at the lateral margin of the body.

The ovary is situated in the middle line, at the posterior border of the ventral sucker, between the testes. Sometimes it is a little in front of, sometimes a little behind the testes. It is globular and has a diameter of 0·09 mm. Alongside the ovary, sometimes dorsal to it, is a very small receptaculum seminis, from which passes a short Laurer's canal. The yolk-glands consist of a small compact mass lying near the ovary, sometimes on its right side, sometimes behind it. The uterus lies almost entirely behind the ventral sucker, although one convolution is thrown up along the right side of the sucker. On the left side and in front of the sucker the uterus runs into a highly developed and very muscular vagina. This lies behind the cirrus-pouch and is considerably inflated. The eggs, as in all the members of the family, develop rapidly

and a completely formed and actively motile larva is present long before the eggs are laid. The egg-capsules vary greatly in size, those in the initial part of the uterus measuring about 0·016 × 0·012 mm., while in the terminal part they measure 0·036 × 0·018 mm. The egg-capsules are, thus, less than half the size of those in *Zoogonoides viviparus*.

Of the systematic position of this form there can be little doubt. It is without question a member of the family Zoogonidae, and of the subfamily Zoogoninae, bearing a close resemblance to the genera *Zoogonus* and *Zoogonoides*. So closely, indeed, does it resemble the latter, that I was at first inclined to include it in this genus. Odhner's (1911) revision of the family, however, distinctly indicates that it is an intermediate form between *Zoogonus* and *Zoogonoides*. I have on that account made it the type of a new genus. Its generic characters may be summed up as follows: resembling *Zoogonoides* but differing in having the cirrus unarmed, in having a very highly developed vagina, and in possessing much smaller egg-capsules.

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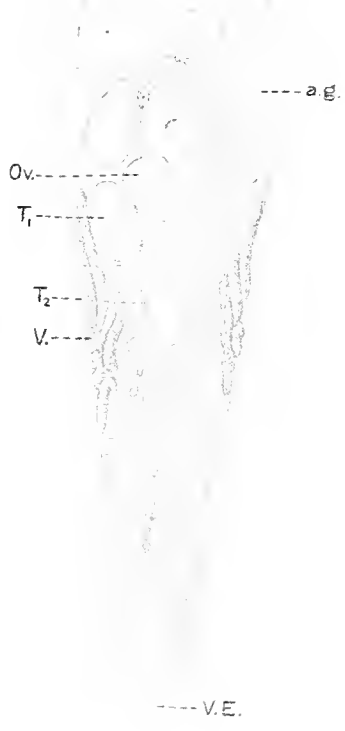

Fig. 1.

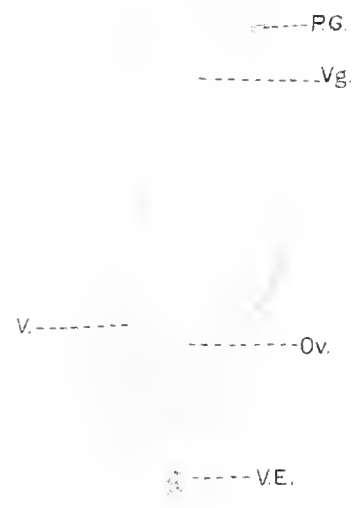

Fig. 2.

M. Rhodes, del.

Fig. 1. *Ancylocoelium typicum.* Ventral view (× 50). a.g., accessory genital sac; Ov., ovary; T_1, T_2, testes; V., yolk-glands; V. E., excretory vesicle.

Fig. 2. *Zoonogenus vividus.* Ventral view (× 65). Ov., ovary; P. G., genital aperture; V., yolk-gland; V. E., excretory vesicle; Vg., vagina.

THE SENSORY PERCEPTIONS OF *ARGAS PERSICUS* (OKEN).

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(With 2 Diagrams.)

Introduction.

Although numerous papers dealing with the anatomy and general biology of ticks have appeared during the last few years, up to the present Lahille (1905) is the only writer who has attempted to throw any light upon the sensory perceptions of the Ixodoidea. However, Lahille's notes on the behaviour of *Boophilus annulatus*, the female of which was the only tick with which he experimented, are somewhat imperfect, although he was the first to suggest the true function of "Haller's organ"—the only sensory organ which is known to occur in all stages of every member of this group. Last year, in this laboratory, Miss D. Jordan Lloyd commenced working on this subject, under Prof. Nuttall's direction, and performed numerous experiments, without, however, arriving at any definite conclusions as to the behaviour of the ticks. Being unable to complete the work, none of these results were published but when, subsequently, we took up this investigation Miss Jordan Lloyd very kindly allowed us to make full use of her notes. It was with the object of clearing up some of the ambiguities regarding the sensory perceptions of ticks and especially to determine the function of "Haller's organ," that the present investigation was commenced.

We have mainly worked with the fowl tick, *Argas persicus* (Oken, 1818), as owing to the kindness respectively of Prof. E. Marchoux, Institut Pasteur, Paris, and Dr A. Balfour, Khartoum, a plentiful supply of this material was placed at our disposal. Moreover, this tick lends itself for experimental purposes more readily than most other species owing to the ease with which it may be reared and the rapidity of its feeding. We have, however, also experimented with *Ornithodorus moubata* (Murray, 1877) and *Hyalomma aegyptium* (Linn.) which were received by Prof. Nuttall from Africa.

Reactions to light.

It is well known that the majority of the Argasidae walk away from light and appear to search out dark corners in which to hide themselves. Lahille describes *Boophilus* as being strongly negatively phototropic and gives a series of diagrams showing the paths taken respectively by each of a series of adult ticks. These diagrams, however, whilst demonstrating that the ticks on the whole walk away from the light, thus suggesting negative phototropism, also show that the ticks deviate considerably from the straight direction, and make one hesitate in using the term phototropism. It is far more probable that the reaction to light is a combination of negative phototropism and a perception of any difference in the intensity of the illumination, and this view is in accordance with the results which we have obtained in the following experiments with *Argas persicus*.

I. Phototropism, employing daylight.

In all cases the reaction of the ticks to direct daylight was tested in the following manner. A large white sheet of paper was placed in front of a window and a line was ruled across it, at right angles to the direction of the rays of light. Twenty of the ticks to be examined were arranged along this line and allowed to walk freely in any direction. The results were as follows:—

(*a*) 30 unfed larvae. In this case all walked away from the light.

(*b*) 20 fed larvae. All negatively phototropic.

(*c*) 20 unfed first-stage nymphs negatively phototropic.

(*d*) 20 fed „ „ „ „ „

(*e*) 20 adults. In this case although every tick walked off the edge of the paper at a point behind the line from which they started,

they did not react so definitely as the immature stages. Some individuals walked directly away from the light, but in a few cases for a short distance they approached the source of illumination, eventually, however, turning round and walking in the opposite direction. The above experiments (*a–e*) were repeated ten times with similar results in each case.

I a. Phototropism, employing artificial light.

The above series of experiments were repeated employing electric light as the source of illumination. White, red and yellow lights were used, respectively, and the results were practically identical with those obtained with daylight. If anything in these experiments the negative phototropism was rather more marked.

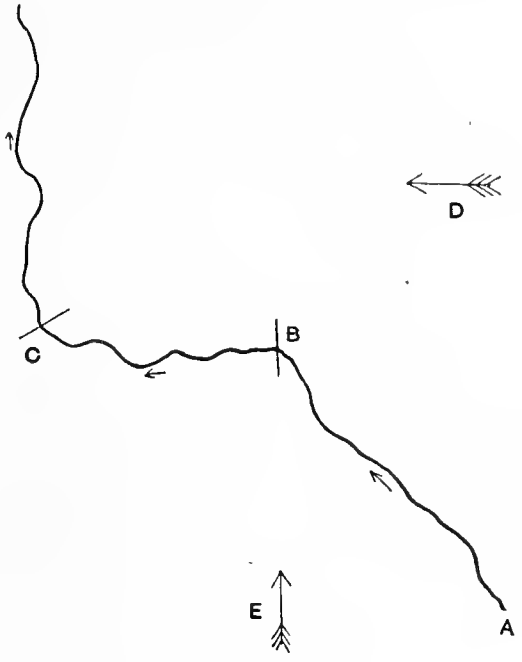

Diagram 1.

In addition, several attempts were made to cover various parts of the tick with substances opaque to light, in the hope of ascertaining whether the whole surface of the tick was equally sensitive. Unfortunately we were unable to find any method of covering the tick's surface, either in part, or whole, without seriously interfering with its movements. Therefore it was attempted to solve this question by exposing the ticks to the action of light coming from two different sources at right angles to each other.

The path taken by each tick under the influence of both lights simultaneously and of each light separately, closely approximated that of the tracing of the track represented in Diagram 1.

In this diagram *D* and *E* show respectively the direction of the rays of light coming from each source. The tick was placed at the point *A* and up to the point *B* was exposed to the action of both lights. It will be noticed that the path taken by the tick is the resultant of the actions of the two beams of light coming from *D* and *E* respectively. When it reached the point *B* the light coming from *E* was shut off by means of a screen and at once the tick altered its direction and walked directly away from the only remaining light coming from *D*. It was allowed to walk in this direction until it reached *C*, at which point the position of the screen was altered so as to again expose the tick to the action of the rays coming from *E* whilst intercepting those from *D*. Here again the tick altered its direction until it walked directly away from *E*. Similar results were obtained with both nymphal and adult stages.

These experiments show that the light acts equally on both sides of the tick and as, with the exception of Haller's organ (*vide infra*), no sensory organs appear to be present in *A. persicus* it may reasonably be assumed that the light acts on all parts of the surface. This view is supported by the fact that the sensitiveness to the action of light is most marked on the larval and early nymphal stages, in which the integument is very thin, and least evident in the adults, these being protected by a thicker integument.

II. Perception of differences in the intensity of light.

Although in the foregoing experiments it has been shown that these ticks exhibit the property of negative phototropism, probably the most important factor which influences them in their reactions to light is a perception of differences in the intensity of the illumination.

This property may easily be demonstrated by placing a large number of ticks in a vessel, part of which is shaded from the light, when the ticks congregate in the shaded regions, with the exception of the unfed larvae which do not react very definitely.

This perception of differences in intensity is much more strongly developed than the negative phototropism as may be shown by the following experiment (Diagram 2).

In this diagram the top arrow represents the direction of the horizontal rays of light which acted during the whole experiment. In addition below the line XY the paper was brilliantly illuminated from above. Five ticks A, B, C, D, E, were placed on the paper and allowed to walk freely in any direction. In this case they all walked directly away from the source of light until they reached the area of bright illumination from above (XY). In every case the tick retreated, in

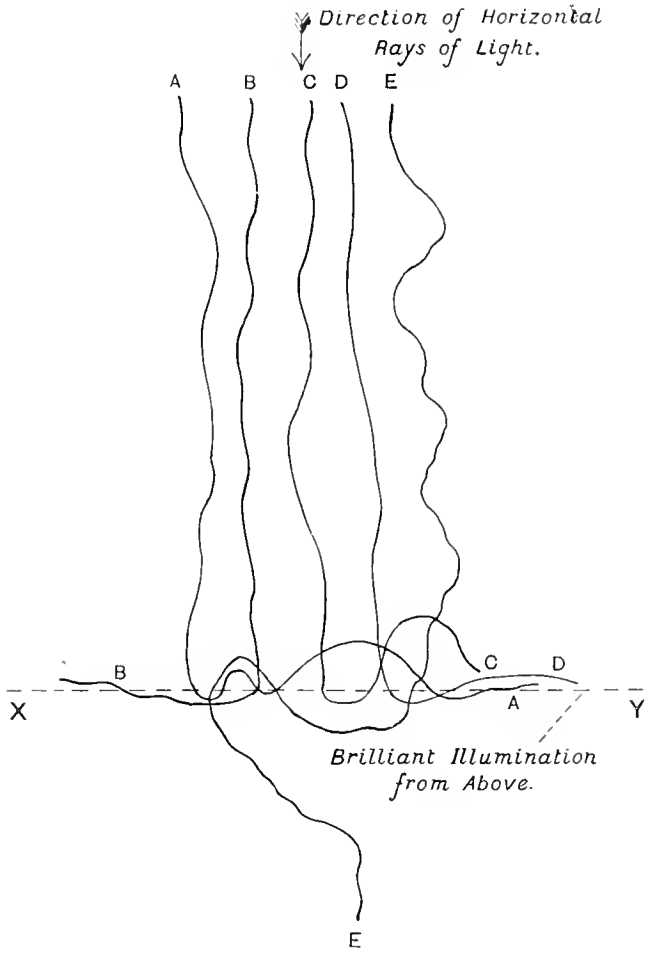

Diagram 2.

opposition to the action of the horizontal rays, and endeavoured to avoid the region of brilliant illumination. In only one case (E) did the tick after coming into the region for a second time, attempt to cross it, and shortly after its entrance the action of the horizontal rays again became apparent. In every other case the ticks skirted along the edge of the illuminated area and would not enter it.

In this case two conflicting influences are at work. First, the action of the horizontal rays tending to drive the tick in a direction away from the source of light owing to the negative phototropism. Secondly, the difference in the intensity of the illumination after the line *XY*, the perception of which is so strongly developed in these animals, that when they entered the region of brighter illumination, although the latter by reason of its vertical direction could not have any phototropic effect, the ticks retreated from this area, preferring to remain in the less brightly illuminated part of the paper even in opposition to their negative phototropism.

Reactions to gravity.

The reactions of *A. persicus* to the action of gravity are not well marked. The experiments were conducted in a dark room in order to avoid the influence of light. Several of the ticks to be examined were placed in the middle of a large sheet of paper and the latter was then supported vertically. In all stages it was found that roughly speaking the unfed ticks merely scattered in all directions whilst the fed ones practically always walked towards the bottom of the paper. This effect was most marked in the case of the fed larvae and first stage nymphs respectively.

Reactions to contact with solids.

At all stages of development, *A. persicus* exhibits a well-marked tendency to bring as much of its surface as possible in contact with external foreign bodies. This positive thigmotropism is the reason why ticks push themselves under any loose solid particles, or into any crevices they meet. The most convenient way of demonstrating this property is by placing a number of ticks into a large glass vessel containing one or two pieces of filter-paper. If the vessel be left in a dark room for a few hours the ticks will all be found congregated underneath the pieces of paper. This experiment may be varied by using V shaped pieces of paper when the ticks will be found congregated in the angles of the Vs, this being the position in which most of their surface is in contact with other solids.

Reactions to heat.

The fact that *Argas persicus* is attracted by radiant heat was first noticed in one of our earlier experiments undertaken in order to determine whether these ticks reacted to differences in the intensity of light. In this experiment a large number of larvae were put into a closed glass tube half of which was covered with black paper. This was then exposed to an electric light, the tube being arranged transversely to the direction of the rays, so that one half was shaded and the other brightly illuminated. It was noticed that the ticks invariably settled down at the junction of the light and shaded parts. At first we were rather at a loss to understand why these animals should always select such a position, for when this experiment was repeated employing daylight instead of electric light, the ticks settled down in the darkest parts of the shaded region and never at the junction of the two portions as in the previous case. It was evident from these results that in the first case we had overlooked some additional factor which was also affecting the parasites. This complicating influence was soon found to be the radiant heat from the electric lamp, and the reason that the ticks settled down at the junction of the shaded and light regions of the tube was because this position combined a maximum of heat with a minimum of light. If the heat element was eliminated it was found that the ticks withdrew into the more shaded regions of the tube.

The effect of radiant heat was also tested employing as the source of heat an incubator at a temperature of about 70° C. If a number of ticks were liberated within about 6 inches of the incubator they all approached it, and as this experiment was performed in the dark, phototropism played no part in the reaction.

A frequent source of error in experiments to decide whether these ticks will settle down in either the warm or cold region of a tube in which they are confined, is the fact that below a temperature of about 15° C., the ticks at once come to rest and remain dormant. As a result, if a tube, in which ticks are evenly distributed, be exposed at any part to temperatures below 15° C. a certain number will always be found in this region owing to their becoming torpid on account of the low temperature.

The effect of moist heat in regard to the feeding of the tick is discussed later.

Olfactory sense.

Lahille is the only writer who has hitherto published any definite experiments relating to this subject. He found that when barriers of a solution of "Sarnol" of either 1 % or 100 % were placed across the track of a moving *Boophilus* (♀)—the only tick with which he experimented—the parasite turned aside before having actually touched the liquid. On the other hand, ticks which had suffered amputation of the first pair of legs, thus being deprived of their Haller's organs, were not checked in their paths by barriers of 1 % Sarnol, but hesitated slightly before crossing barriers of pure Sarnol. From these experiments Lahille concluded that ticks possess an olfactory sense residing in Haller's organ, and that in addition the whole body is sensitive to very strong odours such as pure Sarnol. Miss Jordan Lloyd performed various experiments with gorged nymphs of *Rhipicephalus bursa* which seemed to confirm Lahille's results. In addition to amputation of the Haller's organs, she also employed the method of occluding them by means of a solution of Canada Balsam in benzol. Although the experiments with *R. bursa* seemed to demonstrate the existence of an olfactory sense in these ticks, the results obtained with *Argas persicus* were very indefinite.

Our experiments on the sense of smell have been made almost entirely on *Argas persicus*, and at first we employed the method of placing barriers of various odoriferous substances across the paths of moving ticks. Later this method was abandoned as it also brought in various conflicting factors, such as touch, heat etc., which interfered with a true interpretation of the results. The method we finally adopted was to employ merely the vapours of various odoriferous substances in the manner which will be described below.

I. Experiments with normal ticks.

In the first case are given the results obtained by the use of barriers of various odoriferous liquids and solids. In each case control experiments were made by placing barriers of either water or sand across the paths of moving ticks in order to determine whether they were affected by the sense of touch. The method adopted in each experiment was as follows:—

A definite number of ticks, usually nymphs and adults, were placed in the middle of a large sheet of filter paper, arranged in front of

a window so that the ticks walked in a definite direction away from the light. A crescentic barrier of the substance to be tried was arranged across the filter paper in such a position that in walking away from the light the ticks were bound to come in contact with it. The following results were obtained :–

(*a*) Experiments with liquids[1].

(i) Water. 50 second stage nymphs were employed. On reaching the barrier all hesitated, but finally 46 crossed whilst 4 skirted round the moist area.

(ii) Ammonia. The same ticks were employed for this experiment. In this case the ticks stopped *before* reaching the barrier, but after some hesitation 45 crossed whilst 5 skirted the barrier. It should be added that by the time the ticks crossed the liquid the smell of ammonia was very faint.

(iii) Phenol. The results obtained in this case were precisely similar to those obtained with ammonia.

(iv) Glacial Acetic Acid. Reaction similar to the preceding.

(v) Clove Oil. 50 second stage nymphs were employed. All showed a well-marked reaction to the smell. Before reaching the barrier all the ticks retreated, 17 skirting completely round the liquid. Finally, however, 33 crossed the barrier but with obvious distaste.

(vi) Olive Oil. 50 second stage nymphs were employed. In this case the ticks did not retreat until after they had actually marched into the oil. Eventually 36 crossed the barrier, whilst 14 went back and skirted it.

(*b*) Experiments with solids.

(i) Sand. 50 second stage nymphs were employed. All except 3 ticks walked directly across the sand without any hesitation.

(ii) Naphthalene. The same ticks were again employed. In this case all except 3 crossed the barrier but exhibited slight hesitation before coming in contact with it.

(iii) Ammonium Carbonate and Keating's Powder respectively, gave similar results to naphthalene.

In all the previous experiments it was possible that the ticks had been unable to display their full reactions to the various odours owing to the preponderating influence of their negative phototropism tending

[1] In all these experiments the filter paper was merely moistened with the liquid.

to drive them across the barriers. Accordingly the foregoing experiments were repeated in the dark but the results obtained were practically identical.

From these results it would appear as if *Argas persicus* were only slightly susceptible to the effects of smells, but on employing different methods in the investigation of this subject very different results were obtained.

(*c*) *Experiments with vapours.*

Finally the following experiments were devised in which the complicating factors of touch and differences in temperature were eliminated. A definite number of ticks were placed on a level sheet of filter paper which was supported about three inches above the bench. By this means it was possible to introduce beneath the filter paper wide-mouthed bottles containing the various odoriferous substances. The ticks could therefore be exposed to the action of the vapours that rose through the filter paper without having to moisten the paper or place solid substances across their paths. The results were very definite and showed that *Argas persicus* undoubtedly possesses a well-developed sense of smell.

The vapours of a large number of substances were tested and in practically every case the ticks proved susceptible to the odours. The following substances were tested:—ammonia, butyric acid, naphthalene, clove oil, phenol, and the faeces of a fowl.

The ticks proved very susceptible to the effects of the vapours of ammonia and clove oil respectively. Immediately their front legs entered the region of the vapour the ticks precipitately retired and without exception avoided it. In the case of phenol, naphthalene, and butyric acid, respectively, the effects were slightly different. On entering the vapour the tick stopped and then stood still for a short time, waving its front legs in the air much in the same way that many insects move their antennae. Sometimes after one or two uncertain movements the ticks moved backwards away from the odorous region but in other cases they marched across it.

In the case of all these substances the vapours are more or less irritating to any sensitive part of the body and therefore it was possible that these reactions did not indicate any sense of smell but were merely the result of the general irritating effects of these substances. Accordingly, an experiment was made employing the odour of fowl faeces which could not possess any general irritant properties. The

effects were most marked. The ticks stopped suddenly on entering the region of the odour and, after waving their front legs, quickly retreated.

Having proved the existence of a sense of smell in this species of tick we then proceeded to determine in which part of the body it was localised. Following the experiments of Lahille and Miss Jordan Lloyd and also from the peculiar manner in which the ticks moved their front pair of legs on entering a vapour, there was no hesitation in selecting Haller's organ as the probable seat of the olfactory sense and experiments were made accordingly.

II. Experiments on ticks without Haller's organ.

In order to make certain that this organ was removed, the simple method of amputating the terminal joints of the first pair of legs was adopted. The tick very quickly recovers from the operation but whenever possible the amputation was performed the day before the animal was required for experiment. The method of occluding the Haller's organs by means of various substances was not employed, as with this method it is never certain that the organ is entirely covered and thus rendered functionless.

As the experiments with odoriferous liquids and solids had given such indefinite results, they were not repeated in the present series of experiments but we proceeded directly to ascertain how these ticks without Haller's organ reacted to vapours. Accordingly the last series of experiments was repeated, employing ammonia, clove oil, and fowl faeces respectively, the three substances which had produced the most marked effect on the normal ticks. The results were very striking. In the case of ammonia the mutilated ticks seemed unaware of its presence until they were in the middle of the region of vapour; even here they only seemed to be just aware of its presence in spite of the fact that the smell of ammonia was very powerful, there being a jar of strong liquid ammonia immediately underneath the filter paper. When clove oil was substituted for ammonia the ticks appeared unaffected by it, although some effects might have been expected as in the case of ammonia, because of its irritant properties. In the case of the fowl faeces all the ticks walked through the odorous region without any hesitation.

The foregoing experiments prove that *Argas persicus* possesses a definite olfactory sense, which is situated in Haller's organ.

The function of Haller's organ and the means by which a tick finds its host.

The peculiar sensory structures known as "Haller's organs" were first described by Haller in 1881. On account of their superficial resemblance to the auditory sacs present on the antennules of various Crustacea, he supposed that they were auditory in function and actually described the existence of otoliths in one of the cavities of this organ. Lahille, in 1905, was the first to question Haller's interpretation of their function and as a result of his experiments with the female of *Boophilus annulatus* suggested that they were olfactory organs. Previously, however, Batelli (1891) suggested that they might serve as a means of perceiving at a distance the hosts on which the ticks feed, but this author brings forward no evidence in support of his hypothesis. Nuttall, Cooper and Robinson (1908) were the first to give an accurate description of the structure of this organ. In *Haemaphysalis punctata* it consists of a minute cavity, about 65 μ in diameter, containing sensory hairs, and is associated with a specially modified region of the hypodermis. It is always situated on the external dorsal surface of the tarsus of the first pair of legs. As we have shown, the function of this organ is certainly olfactory, but it would be premature to state that this is its only function. The presence of Haller's organ, however, is essential to the tick in the discrimination of its hosts as may be shown by the interesting results of the following experiments.

Whilst conducting the experiments on the effects of odorous vapours on both normal ticks and those in which the forelegs had been amputated, we also tried the effect of placing a vessel of hot water beneath the filter paper on which the ticks were walking. In the case of the mutilated ticks all showed themselves sensitive to the combination of warmth and moisture, and not only this but many of them inserted their mouth parts and endeavoured to feed through the filter paper, showing that they were unable to discriminate between warm moist filter paper and a fowl, their normal host. On the other hand normal ticks, although susceptible to the warmth and moisture, never showed the least inclination to feed, although they were equally as hungry as the others. These results suggested to us a method by means of which ticks might be made to feed on any desired liquid, and after various

experiments the following procedure was found to be the most satisfactory :—

A piece of glass tube about 3 inches in length and 1 inch in diameter was closed at one end by a piece of diaphragm from a freshly killed rat. This membrane was found to be the most suitable as it is of convenient strength and thickness to allow the tick to anchor itself for purposes of feeding. The tube was then completely filled with the liquid on which the ticks were required to feed and the open end closed by means of plasticine, care being taken to exclude any air bubbles. By means of its plasticine base, the tube was fixed vertically to the bottom of a saucepan, which was then filled with water to within about $\frac{1}{8}''$ below the level of the membrane closing the upper end of the tube. The whole was then heated to about 42° C. and kept constant at this temperature. Ticks from which Haller's organ had been removed, were now placed on the membrane and allowed to anchor themselves, which was usually accomplished without much hesitation, and the ticks then proceeded to gorge themselves on the fluid contained in the tube. In the first case a mixture of a trace of rat's blood in sodium citrate solution was employed; the ticks having fully gorged on this almost transparent liquid presented a very curious translucent appearance. These ticks, however, did not emit any coxal fluid and were found dead the following day. In a second case, liquid gelatin was employed which was also ingested though less readily than the previous liquid. Ticks were also fed on normal 0·8 % NaCl solution without the addition of any other substance.

Having obtained these results with *Argas persicus*, we went on to experiment with other species of ticks, from which Haller's organs had been removed. *Ornithodorus moubata* behaved in exactly a similar manner to *Argas persicus*, and fed on liquid gelatin. On the other hand, it was found that *Hyalomma aegyptium* although they attempted to anchor themselves were unable to do so owing to the slippery nature of the membrane. Accordingly a piece of ordinary rat skin with the hair outside was used instead of the diaphragm and it was then found that the ticks readily anchored themselves and commenced to feed on the normal salt solution contained in the tube.

These experiments demonstrate that in the absence of Haller's organs a tick loses all power of discriminating its host. In finding a host the tick seems to be mainly governed by two factors. First, the effect of heat, the perception of which is distributed over the general surface of the body, and secondly the sense of smell, located in Haller's

organ. It seems doubtful whether ticks possess any gustatory organs as in our experiments the ticks fed on both normal salt solution and liquid gelatin, substances which could not present the slightest resemblance to blood as regards taste.

Summary.

1. All stages of *Argas persicus* are negatively phototropic. In addition, they are also susceptible to pronounced differences in the intensity of the illumination and select the darker places.

2. In the gorged state, the ticks are slightly positively geotropic. In the unfed state, this property is not developed.

3. At all stages the tick endeavours to bring as much of its surface as possible in contact with its surroundings.

4. *A. persicus* is attracted by heat.

5. In all stages an olfactory sense is well developed.

6. "Haller's organ" is olfactory in function and constitutes a means by which a tick is able to recognise its host. By depriving ticks of this organ it is possible by suitable means to cause them to feed on media other than blood, thus showing that a sense of taste is absent. *Argas persicus*, *Ornithodorus moubata* and *Hyalomma aegyptium* have all given similar results with regard to the latter point and we believe that this constitutes a method by which perhaps other blood-sucking arthropods, after being deprived of the organ or organs necessary for the recognition of their hosts, may be made to feed on any desired medium.

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GASTROTHYLAX BUBALIS, N. SP. WITH A FEW NOTES ON THE GENUS *GASTROTHYLAX* (POIRIER).

By J. ALEXANDER INNES.

(*From the Zoological Laboratory, University of Aberdeen.*)

(With 8 Text-figures.)

The trematode described in this paper was found by Dr Alexander Brown, British Central Africa, in the stomach of a Rhodesian hartebeest (*Bubalis* sp.). About 120 specimens were collected and placed at my disposal for investigation by Dr John Rennie, Lecturer on Parasitology in this University, to whom I am much indebted for the use of the interesting material. The specimens proved to belong to a new species to which the name *Gastrothylax bubalis* has been given. Although a fuller knowledge of recorded species rather than the recovery of new trematodes is to be desired, it is hoped that the description of this parasite may be interesting from the fact that the genus includes only ten other known species.

Technique. The parasite was of such dimensions that a single specimen could not be mounted whole as an entire preparation, and therefore several sets of serial sections were prepared. In spite of the thick, tough cuticle it was found that the sections cut quite well at a thickness of 10 μ, if the time taken in the various paraffin baths was considerably shortened. If the process of embedding took longer than two hours the specimens became very brittle and serial sectioning was practically useless. Both transverse and longitudinal serial sections were made, but the latter were the more valuable. Staining with haematoxylin and eosin gave good results but for all purposes staining in bulk with paracarmine (Mayer) for three days, and then differentiating

by washing in several changes of 70% alcohol to which a little 1% solution of ammonium chloride had been added, was found to answer best. Owing to the imperfect method of fixation the finer histological details were not examined. In tracing the various ducts, etc., rough reconstructions were made.

The determination of the genus, *Gastrothylax*, is made at once when a transverse section reveals the generic characteristic, viz., a spacious pouch, atrium or belly-pocket ("Bäuchtasche"), which opens anteriorly by the atrial pore. A specimen cleared in clove oil confirms the determination by showing the position and number of testes, the branched gut and the large terminal posterior sucker.

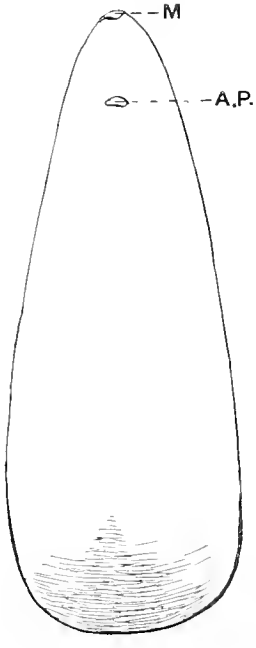

Fig. 1.

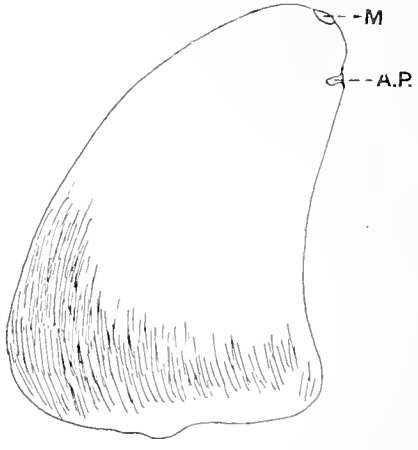

Fig. 2.

Fig. 1. Outline of *G. bubalis*. *M.* mouth. *A.P.* opening of atrium.

Fig. 2. Outline of contracted specimen of *G. bubalis*. *M.* mouth. *A.P.* opening of atrium.

External anatomy. The parasite is conical in shape with blunt anterior end, and has a length of from 8–10 mm. and its posterior diameter is about 3 mm.[1] The trematode is of a white or light gray colour and none of the specimens showed the characteristic red coloration. The form varies from an almost cylindrical shape, in fully expanded

[1] Six specimens gave the following measurements in mm.: $11{\cdot}5 \times 3{\cdot}75$; $12{\cdot}5 \times 4$; $10{\cdot}0 \times 4{\cdot}5$ and $9{\cdot}0 \times 4$ (contracted specimens); $8{\cdot}75 \times 4{\cdot}5$ (contracted, pear-shaped specimen); $7{\cdot}0 \times 3{\cdot}75$ (small specimen, slightly contracted).

examples (Fig. 1), to a short stumpy cone with a highly wrinkled surface (Fig. 2). In contracted examples the posterior sucker is reduced to about one third the diameter of the terminal area and retracted within it. A cross section through any region of the body appears to be circular. The mouth opens anteriorly (Fig. 1, *M.*) exactly at the apex of the cone, and is surrounded by a mass of muscular tissue. It may be used sometimes for adhesion. Oral papillae are present.

The transverse opening of the genital atrium lies a little behind the mouth in the mid-ventral line (Fig. 1, *A.P.*) and is easily made out in all specimens. It is lined internally with papillae.

The large posterior sucker is placed terminally and when expanded occupies almost all the posterior portion of the parasite (Figs. 3, 4 and 5, *P.S.*). It seems to have a slight inclination towards the ventral surface. The ventral side is straight, or slightly concave. The dorsum is distinctly convex.

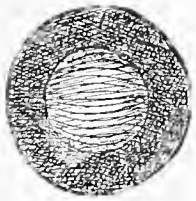

Fig. 3.

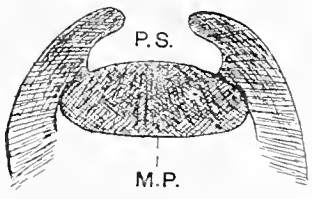

Fig. 4.

Fig. 3. Terminal sucker of *G. bubalis.*

Fig. 4. Longitudinal section through posterior end of *G. bubalis.* *P.S.* posterior sucker. *M.P.* muscular part.

Internal anatomy. The alimentary canal consists of the anterior terminal mouth, a muscular pharynx, and a short oesophagus which bifurcates on a level with the middle of the prostate gland, into the two limbs, or pockets of the gut. These limbs are blind sacs and pass down on either side of the atrium (Fig. 6, *L.G.*). They pass dorsal to the testes and terminate on a level with the middle of these organs (Fig. 5). The gut branches show varied convolutions. In some species of *Gastrothylax* the gut pockets do not reach the testes. The genital atrium is large and roomy and expands towards the posterior end where it terminates just above the anterior boundary of the testes. It is triangular in cross section with a ventrally directed apex (Fig. 6, *At.*). In certain sections through the atrium clots of organic matter have been seen, while in one case there was a single ovum. The physiology of this organ is at present unknown. It opens to the exterior at the anterior end on the ventral surface and in this region also receives the genital opening (Fig. 5, *A.P.G.*).

The reproductive system is fairly complex and situated almost entirely at the posterior end, in what may be termed the genital area, *i.e.* between the base of the atrium and the posterior sucker. In all the other species, except *G. mancupatus,* the reproductive organs are placed more laterally.

The male system consists of a pair of large round testes,—which do not extend close to the body-wall of the parasite, nor into the atrial cavity—two vasa efferentia, and a single long vas deferens opening along with the vagina at the genital aperture. The vas deferens and vagina run along the dorsal fork of the atrium (Fig. 6, *Vd.*, *Vg.*). The two vasa efferentia come one from each testis and unite to form the vas deferens.

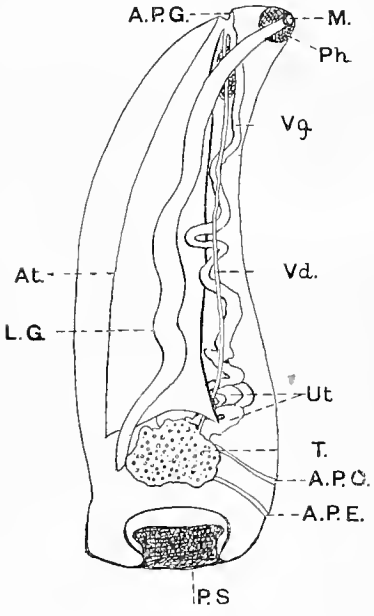

Fig. 5.

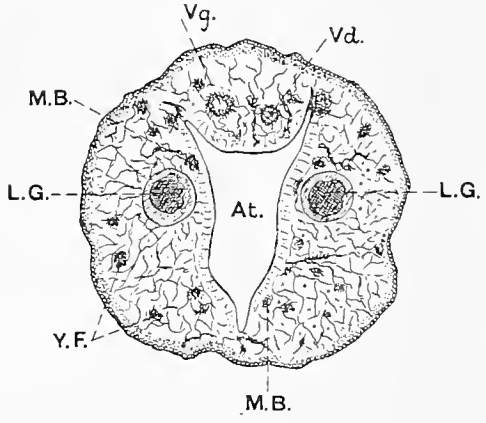

Fig. 6.

Fig. 5. Diagrammatic longitudinal section of *G. bubalis*. *M.* mouth. *A.P.G.* genital and atrial apertures. *Ph.* pharynx. *Vg.* vagina. *Vd.* vas deferens. *At.* atrium. *L.G.* gut-limb. *Ut.* uterus. *T.* testes. *A.P.C.* aperture of Laurer's canal. *A.P.E.* aperture of excretory vesicle. *P.S.* posterior sucker.

Fig. 6. Cross section through middle region of *G. bubalis*. *At.* atrium. *L.G.* right and left gut limbs. *Vg.* vagina. *Vd.* vas deferens. *M.B.* muscle bundles. *Y.F.* yolk follicles.

The female system consists of ovary, shell gland, oviduct, uterus and vagina. In this species the shell gland and ovary are very close together, the latter is about the same size as the shell gland, and the duct from the shell gland almost immediately joins that of the ovary (Fig. 7).

Another feature in this species is the position of these organs, which are situated centrally between the two testes, and between the atrium and posterior sucker. The uterus is much convoluted but it is not "clumped" (geknäult). It was filled in parts with ova measuring about $110 \times 60\ \mu$. In certain regions the uterus seemed to be surrounded by a thick syncitium. The vagina opens anteriorly at the genital aperture.

The excretory vesicle of this species is unique both in position and outline. It is situated immediately in front of the posterior sucker and behind the shell gland, while in the other species it has a more or less

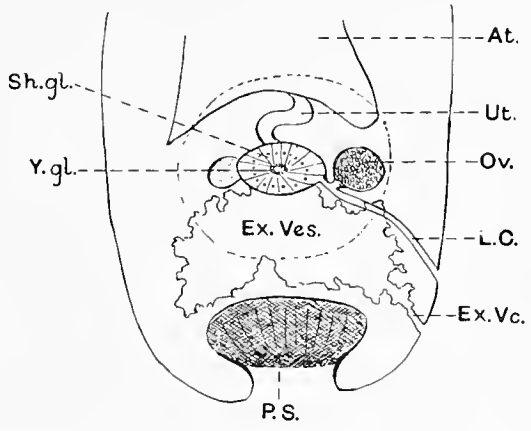

Fig. 7. Diagram showing relation of the genitalia in *G. bubalis*. Position of testes shown by dotted lines. *At.* atrium. *Ov.* ovary. *Sh.gl.* shell gland. *Y.gl.* yolk gland. *Ut.* uterus. *L.C.* Laurer's canal. *Ex.Ves.* excretory vesicle. *Ex.Vc.* canal of excretory vesicle. *P.S.* posterior sucker.

lateral position. The great irregularity of its outline is also striking. The canal of the excretory vesicle is short and does not unite with Laurer's canal as in *G. wenyoni* (Leiper, 1908) but opens separately below it (Fig. 7, *Ex. Vc.*).

The musculature of the parasite seems to be concentrated in the suctorial regions at either extremity and there seems to be as much muscular tissue at the posterior end as there is in the rest of the body. Thin bands of muscle fibres run longitudinally, but there are few circular muscles in the middle of the body. The yolk follicles are united into small groups and form a kind of plexus. They are irregularly scattered throughout the body, being placed both dorsoventrally and dorsolaterally (Fig. 6, *Y.F.*). Like *G. compressus*, the general tissue of the animal is very loose, parenchymatous and spongy.

The parasite is believed to occasion no inconvenience to its host.

Notes on the genus Gastrothylax.

Creplin in 1847 discovered the first species of *Gastrothylax*, which he found in the stomach of the Zebu (*Bos taurus indicus*). He classed his parasite as *Amphistomum* (Rud.) *crumenifer*. *G.* (*Amphistomum*) *crumenifer* is therefore the first known species. In 1882, Poirier (1883) discovered two new helminths which he found did not agree completely with the characters of *Amphistomum*, and for these he created a new genus called *Gastrothylax*. The two parasites described by him were *G. elongatus* and *G. cobboldi* from the stomach of *Palonia frontalis* of Java. *G. elongatus* has since been found in Ceylon (*Bos taurus*) and there is one specimen in the Berlin museum from an African cow (*Bos sp.*). *G. cobboldi* has also been found in China (from *Bos taurus*). In 1896 Looss, of Cairo, found a single example of a new species, *G. gregarius*, in *Bos bubalis* of Egypt. In 1898, Brandes described two other species, *G. compressus* and *G. spatiosus*, from *Bos taurus* of Africa. Leiper (1908) quotes these as occurring, the former in *Bos taurus indicus* of India, and the latter in *Bos taurus* of Arabia. Fischoeder in 1901 drew up a new classification of the *Amphistomidae* (Montic. 1888). He changed the family name to *Paramphistomidae*, and divided it into two sub-families, *Paramphistominae* and *Cladorchinae*. The first of these was divided into three genera, *Paramphistomum* (*Amphistomum* Rud.), *Stephanopharynx* n.g., and *Gastrothylax* (Poirier, 1882). In the same paper he briefly describes three new species, *G. synethes* and *G. mancupatus* from *Bos taurus* of East Africa; and *G. minutus* from *Antilope, sp.* and the bushback (*Tragelaphus scriptus*) of the German Cameroons, Africa. The fullest account of the *Paramphistomidae* is given by Fischoeder (1903) in the *Zoologische Jahrbücher*, where he gives a detailed description of all the known species. He also gives diagrams illustrating the position and relation of the genitalia of almost all the species of *Gastrothylax*. It should be noted that the distribution of this genus is as far as we know peculiarly restricted, for, with two exceptions—*G. crumenifer* and *G. elongatus*—all the species have been found as parasites of African ungulates. In some cases the same species has been described both from Javan and from Chinese cattle (*e.g. G. cobboldi*), and with three exceptions, it seems that the cow (*Bos taurus*) is the normal host of *Gastrothylax*.

The genus *Gastrothylax* is unique in possessing a curious "belly-pouch" (Bauchtasche), ventral pocket or genital atrium, and this character

alone distinguishes it from all other trematodes. It is a blind sac which receives at its anterior end the opening of the genital ducts, and it extends backwards to the regions of the testes where it expands to a considerable size. Roughly speaking one might liken it to a hollow cone lying ventrally in the body of the parasite with its apex placed anteriorly. The atrium is lined with a thin cuticle, and directly external to the hypodermis many thin muscular fasciculi run longitudinally (Fig. 6, *M.B.*). There are no well defined circular muscles. As stated before, the physiology of this organ is not known, but perhaps it may act as a kind of brood pouch or as a receptacle for storing the ova during

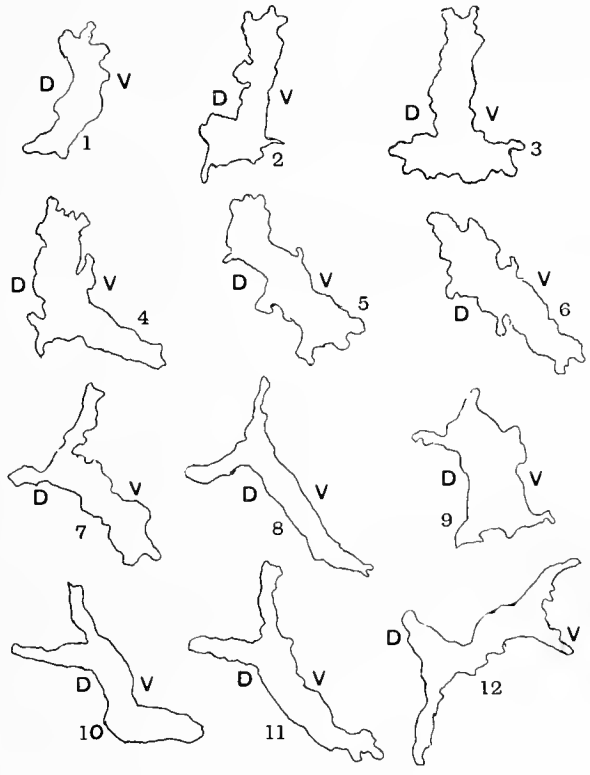

Fig. 8. Changes in the atrium of *G. bubalis*. *D.* dorsal. *V.* ventral.

certain periods. Its exact nature, however, will not be understood until the development has been investigated. The configuration of the atrium on cross section has been used by Fischoeder (1903) as a basis for classification. Thus:

Cross section of atrium triangular with apex directed dorsally—*crumenifer* and *compressus* with pointed apex; *gregarius* and *wenyoni* with bifurcated apex.

Cross section of atrium triangular with apex directed ventrally: *synethes, elongatus, mancupatus, cobboldi* and *minutus.*

Cross section of atrium circular: *spatiosus.*

This system of classification is not quite reliable, for in one specimen of *G. bubalis*, of which serial sections were made, the atrium frequently changed its shape. Fig. 8 shows a few of the changes presented by it in cross section.

The genital organs lie in front of the posterior sucker and may be, as is usually the case, placed fairly near the body wall. They may also be placed nearer the centre. The size of the ovary and of the shell gland varies in the different species, while their proximity to each other also differs. In *G. wenyoni* a notable characteristic is found, and one which is unique in the genus. The duct of the excretory vesicle and Laurer's canal unite to form a single short duct opening to the exterior. The uterus may or may not be clumped. The testes are large, paired organs, situated in front of the posterior sucker, and their position in relation to each other may be specific. The excretory vesicle is placed at the posterior end slightly in front, and at the side of, the large sucker. The size and outline vary. In *G. bubalis* it is exceedingly large and irregular and in one specimen the entire vesicle was filled with a large, glassy, lens-like body which did not stain and which quite changed its shape. Its duct, larger than Laurer's canal, opens separately in all the species except *G. wenyoni.* The gut divides into two a little below the genital aperture, and the level to which the two limbs pass backward is of specific value, since in certain species the gut branches reach only to a third of the body length. The gut pockets are blind sacs and usually exhibit convolutions.

The yolk follicles have no special arrangement, being generally in the form of widely distributed net formations.

The life-histories of the species of *Gastrothylax* are not known, but probably the intermediate host is some freshwater snail and the cycle like that of the sheep trematode—*Fasciola hepatica.*

The following is a brief summary of the known species of *Gastrothylax*, with their distinguishing features other than the shape of the atrium.

(1) *G. crumenifer*, Creplin 1847.

Gut branches are long and tortuous, extending to the level of the testes; testes are placed laterally and close to the body wall. Length 12–18 mm.

(2) *G. compressus*, Brandes 1898.

Gut branches are short, and do not reach the level of the testes. Length 9–11 mm.

(3) *G. gregarius*, Looss 1896.

Gut branches are short and do not reach the level of the testes which are situated close to the body wall.

(4) *G. wenyoni*, Leiper 1908.

Gut branches do not quite reach the level of the testes. Laurer's canal and duct of excretory vesicle unite to open exteriorly by a single aperture.

(5) *G. synethes*, Fischoeder 1901.

Gut branches are long and tortuous, reaching to the level of the testes which are situated close to the body wall. Atrium large. Uterus is clumped nearer the posterior end. Length 7–11 mm.

(6) *G. mancupatus*, Fischoeder 1901.

Gut branches reach the neighbourhood of the testes, which do not lie close to the body wall but whose anterior ends project into the atrium in the form of a cone. The ovary, shell gland and part of uterus are placed between the testes. Length 7–11 mm.

(7) *G. cobboldi*, Poirier 1882.

Gut branches reach back to the posterior sucker, and the testes are placed in the middle line, behind or under each other. Length 8–10 mm.

(8) *G. elongatus*, Poirier 1882.

Gut branches reach only to the middle of the body and pass near each other on the dorsal surface. Testes are placed like those of *G. cobboldi*. Length 13–16 mm.

(9) *G. minutus*, Fischoeder 1901.

This species is recognised by its small size, while the testes are not placed near the body wall but project into the atrium in the shape of a cone. Length 4–5 mm.

(10) *G. spatiosus*, Brandes 1898.

Gut branches only to the commencement of the last third of the body. Testes lie close to the body wall. Length 9–12 mm.

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